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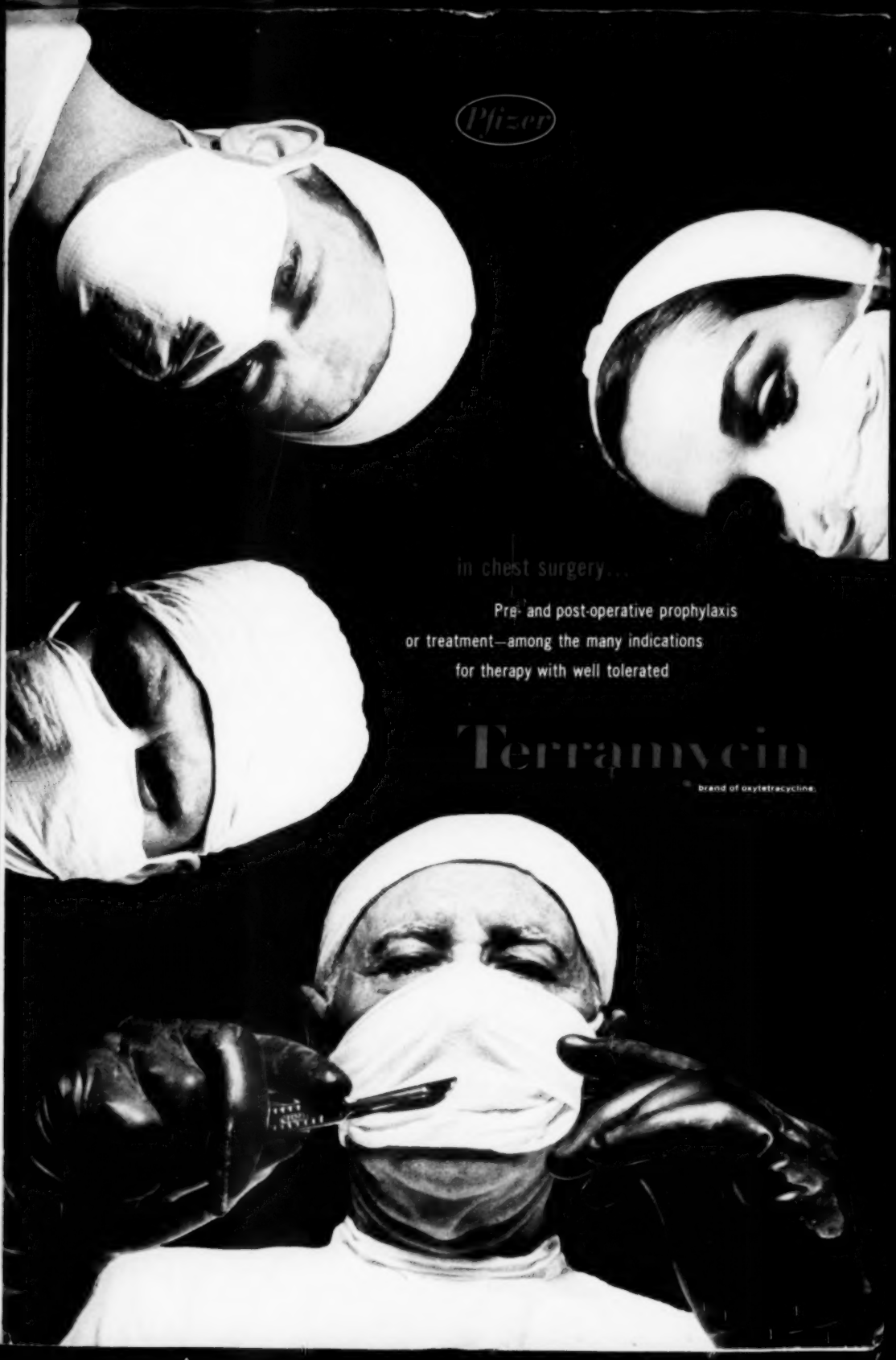
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Although caloric needs in the later decades of life lessen with decreasing physical activity and diminishing metabolic rate, clinical observations[1] corroborated by experimental studies[2] show that protein needs of the aging organism continue at the levels of adequacy in earlier years.[3] For avoidance of protein deficiencies, which the aged are prone to develop,[4] the protein quota of the diet of persons over fifty should be more liberal than is often the practice.[5] In providing this quota, lean meat may well be one of the protein foods of the daily diet.

In the years beyond fifty, as well as before, continuous adequate protein nutrition remains an absolute necessity for maintenance of a normal concentration of plasma proteins and, in turn, a normal osmotic pressure of the plasma.[6] Even more pronounced in the aged than in younger persons are the ill consequences of hypoproteinemia—edema, decreased resistance to generalized infection, retarded bone healing, and poor wound healing.[7] Furthermore, dietary protein is essential for the continuous chemical regeneration of cell protein in the prevention of abnormal tissue wasting, one of the most characteristic and obvious changes in the geriatric patient.[8]

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The editors wish to take this opportunity to express their appreciation to Dr. William B. Tucker, Dr. John D. Steele, Dr. Arthur J. Vorwald, and the late Dr. John D. Adcock[1] for their high quality of performance and diligent service as members of the Editorial Board for the past several years. They will be replaced on the Board as of July 1, 1953, by Dr. Daniel E. Jenkins, Dr. Sidney Raffel, Dr. John W. Strieder, and Dr. Ralph Tompsett.

[1] An obituary notice will appear in an early issue.

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X-RAY THERAPY OF PERIPHERAL TUBERCULOUS LYMPHADENITIS[1]

JOSEPH N. ACETO,[2] KAZUMI KASUGA, AND STEVENS S. SANDERSON

(Received for publication November 28, 1952)

INTRODUCTION

Peripheral tuberculous lymphadenitis frequently causes greater anxiety in a patient than does the threat or actual existence of tuberculous pulmonary disease. This is especially true when the adenitis becomes fluctuant and drains. Even though the adenitis is only one manifestation of a systemic tuberculous infection, most patients will not accept the idea that, given enough time, the adenitis will usually regress and heal without direct intervention as the systemic disease improves. Usually, therefore, some form of specific therapy is attempted.

Before streptomycin became available, several forms of therapy were used for tuberculous lymphadenitis. The most common methods of treatment were surgical excision, surgical drainage by needle aspiration or incision, ultraviolet irradiation, and X-ray irradiation.

The first method is discussed thoroughly in a recent article by C. W. Lester (1) who mentions that surgical extirpation might be the ideal treatment if several conditions could be met; namely, if the infection were not acute and if the general clinical status of the patient were good. He implies technical pitfalls and emphasizes that the patient and the surgeon should be prepared for a long operation. In his article, the treatment is compared by him with a dissection for neoplastic disease, except that important structures are preserved. It seems to the present writers that this type of surgery would be especially difficult for children, who make up a significant group of patients with tuberculous lymphadenitis.

Treatment of tuberculous lymphadenitis by drainage through an incision or by aspiration is frequently complicated by the formation of chronic draining sinuses. With these methods, the results are not predictable and the healing process may take many months or even years.

While the advent of streptomycin provided the first real promise of successful therapy, it has become apparent that, because of toxicity and the emergence of drug-resistant tubercle bacilli, the drug has some limitations in the treatment of

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tuberculosis. Streptomycin should presumably be used in combination with other forms of therapy and in the right case at the right time (2). Ideally, therefore, streptomycin should be used in the treatment of tuberculous lymphadenitis in those patients with concomitant pulmonary or nonpulmonary tuberculosis which requires antimicrobial therapy at that time. The same principles would presumably be applicable to isoniazid and other new drugs. Of the alternative methods available for therapy, the use of X-ray is considered in the present report.

Francis H. Williams, of Boston, is credited as the first to treat tuberculous lymphadenitis with X-ray (3). This was prior to the publication of his book, *The Roentgen Ray in Medicine and Surgery*, in 1902. Most early publications report cases, claiming excellent results, especially in the treatment of draining sinuses. L. I. Kaplan (4) gives an excellent review of the historical background of radiation therapy of tuberculous adenitis. In 1940, Lahey, Hare, and Haug (5) reported satisfactory results in 80 to 90 per cent of the cases of tuberculous lymphadenitis treated by X-ray at the Lahey Clinic. In 1941, H. Hauser (6) reported that the results of X-ray therapy in cervical tuberculous lymphadenitis was satisfactory in approximately 88 per cent of his series. Recently, Lampe and his associates at the University of Michigan (7) revived interest in this mode of therapy by reporting their results in treating thirty-seven patients who suffered from tuberculous cervical lymphadenitis. They were treated with 200 r. (measured in air) to one field daily for four or five days. The average total dosage was 800 to 1,000 r. per field. The patients were given a second course of therapy if healing had not been completed in three months. No more X-ray therapy was given if the second course failed. Approximately 83 per cent of the patients had complete regression following this treatment. Lampe and associates concluded that X-ray therapy would be successful in 75 to 80 per cent of cases and that the use of streptomycin was unnecessary in this situation.

This report from Ann Arbor suggested that further investigation of the use of X-ray in the treatment of tuberculous lymphadenitis might be profitable, and gave impetus to the undertaking of the present study.

METHODS AND MATERIAL

The present report is based upon the study of 65 patients with tuberculous lymphadenitis who received treatment exclusively with technically adequate dosages of X-ray irradiation in the eight-year period from 1941 to 1949. All but 6 were American Indians; the 6 non-Indians were whites.

Of the 65 patients, 29 were male and 36 were female. The youngest patient treated was 2 years old and the oldest was 49 years old. The greatest number of patients, 33, were in the 13- to 19-year age group.

The clinical impression of tuberculous lymphadenitis was based upon the presence of painless swelling of the lymph nodes, usually chronic, without evidence of local nontuberculous infection and without clinical or laboratory findings suggestive of other causes of lymphadenopathy.

Thirty-eight patients had other forms of tuberculosis in addition to the clinical picture of lymphadenitis as described above. Thirty-one of these had active pulmonary tuberculosis. These included one patient with tuberculous salpingitis and one with active Pott's disease in addition to the pulmonary disease. Six patients had associated arrested pulmo-

nary tuberculosis. One of the 38 had tuberculous inguinal lymphadenitis associated with tuberculosis of the knee, without pulmonary tuberculosis.

Twenty-seven patients did not have associated tuberculous infection in other locations. In 4 of these, biopsy and histologic examination of the lesion confirmed the clinical impression of tuberculous etiology. Five patients had positive Mantoux tests as the only additional finding. Eighteen had the previously described clinical picture as the sole basis for diagnosis.

Many of the patients had more than one area involved and treated. The areas sometimes differed with respect to the nature of the lesions and the results of therapy. For the purpose of analysis, each field was considered as a separate unit.

The total number of fields treated was 87. Of these, 79 were cervical and 8 were noncervical. The latter were: draining sinus on the chest wall, three fields; draining sinus in the axilla, three fields; inguinal draining sinus, one field; and enlarged, hard, inguinal nodes, one field.

The technical factors which were constant were the use of 3 mm. of aluminum filter and 8 ma. The KV and r. (measured in air) produced each time varied.

Twenty-three fields were treated with 60 to 90 r. each time, generated by 100 KV. This was labeled *Technique A*.

Fifty-two fields were treated with 114 r. each time, generated by 100 KV, and this was called *Technique B*.

Technique C was the use of 105 to 200 r. each time, generated by 125 to 150 KV. Twelve fields were thus treated.

These variations had not been premeditated but were the result of the installation of a new machine. *Technique A* was the only one used at the beginning of the period being reported. *Technique B* followed and was used exclusively for the longest period of time of the three described. *Technique C* was used for a short period of time toward the end of the total period described in this report.

Therapy was given once a week. The shortest period of therapy was three weeks; the longest, twenty weeks. The average time of therapy was six weeks. The end point of treatment was considered successful if there was disappearance or definite diminution in the size of the hard swellings or cessation of drainage followed by healing.

The observations of the end results used in the statistical analysis were noted either at the time of the final treatment or in a period of one month after the final treatment. Those patients who were still hospitalized for pulmonary tuberculosis were observed for various additional periods of time. Long-range follow-up observations were accomplished in 12 of the patients who did not have associated pulmonary disease and who were discharged immediately after their course of therapy had been concluded. These observations will be discussed in the present report but are not included in the statistical analysis.

RESULTS

Irrespective of the type of lesion or the technique used, the over-all results show that, of the 87 fields treated, the lesions in 44 (somewhat over 50 per cent) exhibited regression or healing. When the type of lesion is considered (table 1), it may be seen that, of 47 fields with fluctuant and/or draining nodes, the lesions of 30 (approximately 63 per cent) regressed or healed. Of 40 fields containing

hard nodes, 14 (approximately 33 per cent) regressed or disappeared. The average treatment time for the lesions which showed a successful result was six weeks.

In an attempt to determine the relative merit of the three techniques, the results were tabulated separately for each technique (table 2). As may be seen, a total of 23 fields was treated with *Technique A*, 12 containing hard nodes and 11 containing draining nodes. Seven of the 12 hard nodes and 6 of the 11 draining nodes were treated successfully.

TABLE 1

OVER-ALL RESULTS OF X-RAY THERAPY OF TUBERCULOUS LYMPHADENITIS

	Number of Fields Treated	Number of Fields *S*	Number of Fields *U*	Per Cent of Fields *S*
Hard nodes	40	14	26	35
Fluctuating and/or draining nodes	47	30	17	63

S indicates successfully treated.
U indicates unsuccessfuly treated.

TABLE 2

COMPARATIVE RESULTS OF THE THREE TECHNIQUES USED IN X-RAY THERAPY OF TUBERCULOUS LYMPHADENITIS

	HARD NODES				FLUCTUANT AND/OR DRAINING			
	Number of Fields Treated	Number of Fields *S*	Number of Fields *U*	Per cent of Fields *S*	Number of Fields Treated	Number of Fields *S*	Number of Fields *U*	Per cent of Fields *S*
Technique A	12	7	5	58	11	6	5	54
Technique B	22	4	18	18	30	19	11	63
Technique C	6	3	3	50	6	5	1	83

S indicates successfully treated.
U indicates unsuccessfully treated.

Using *Technique B*, a total of 52 fields was treated. Of these, 22 contained hard nodes and 30 contained draining nodes. Four of the 22 hard nodes and 19 of the 30 draining nodes were successfully treated.

Using *Technique C*, a total of 12 fields was treated. Six fields contained hard nodes, which in 3 instances regressed or healed. Of 6 fields with draining nodes, 5 were healed following therapy.

Statistical evaluation[3] showed that there was no significant difference in results between hard and draining nodes, using *Techniques A* or *C*. However, the differ-

[3] Ratio of the difference to the standard error of the difference applied to proportions:

$$\sigma_D\,\% = \sqrt{pq\,(1/N_1 + 1/N_2)}$$

where: p is the total percentage of occurence.
q equals $1 - p$.
N_1 is the number in the first sample.
N_2 is the number in the second sample.

ence in results between hard and draining nodes treated by *Technique B* is more than could be accounted for by chance alone.

No significant differences among the three techniques were found in the results of treatment of draining nodes.

Applying the same test to the results with hard nodes, it appears that the results with *Technique B* are significantly poorer than with the other two techniques, while the difference between the results of *A* and *C* is not significant. The reasons for this are not clear. With this one exception, it does not appear that

TABLE 3

DISTRIBUTION OF FIELDS OVER THE RANGE OF TOTAL ROENTGENS OF X-RAY THERAPY

TOTAL ROENTGENS OF THERAPY	HARD NODES		FLUCTUANT AND/OR DRAINING NODES	
	Number of Fields *S*	Number of Fields *U*	Number of Fields *S*	Number of Fields *U*
300 to 399	1	5	7	6
400 to 499	6	2	6	1
500 to 599	0	7	7	2
600 to 699	2	2	2	0
700 to 799	2	4	2	4
800 to 899	0	0	0	0
900 to 999	0	3	2	3
1,000 to 1,099	0	0	0	0
1,100 to 1,199	2	1	1	0
1,200 to 1,299	1	0	1	0
1,300 to 1,399	0	1	1	0
1,400 to 1,499	0	1	1	0
1,800—	0	0	0	1
Total	14	26	30	17

S indicates successfully treated.
U indicates unsuccessfully treated.

the various techniques are greatly different one from the other in the results obtained in the present report.

On review of the results of X-ray treatment of the noncervical diseased nodes, it was found that lesions in only 3 of the 8 fields manifested a successful result.

Regarding the total roentgens of therapy, it was found that the greatest number of successfully treated fields with both hard and draining nodes fell within the range of 400 to 699 r. (table 3).

As mentioned previously, the results analyzed represented the findings immediately after the last treatment or within the ensuing month. Unfortunately, follow-up information is not available in all the cases. The patients who remained in the hospital because of associated pulmonary tuberculosis were observed for varying lengths of time during their stay. Of the patients who were discharged

after the course of X-ray treatment was complete, 12 were seen subsequently. None of the patients seen later had any breakdown of areas which were seen to be healed immediately following treatment. Four fields which were classified as unsuccessful results at the immediate post-therapy observation were healed when seen later. As stated previously, these were not included in the statistical study, since it could not be concluded with certainty that the healing was due directly to the X-ray therapy.

One patient returned several times in a seven-year period for further treatment of a persisting draining sinus on one side of his neck. The lesions on the other side had remained healed since the original X-ray treatment. He had received ultraviolet light irradiation and a course of streptomycin for the still draining sinus, but all to no avail. Seven years after the original X-ray treatment, he was given further X-ray treatment with all the technical factors described earlier in the present report except that 200 r. generated at 125 KV were given every other day for a total of 1,000 r. One month later, it was noted that the draining had stopped and that scar tissue was beginning to form. The original treatment in this case had been with *Technique A* for a total of 456 r.

DISCUSSION

From the results presented in table 1, it may be seen that the tuberculous lesions in more than 6 of 10 fields with fluctuant or draining nodes healed after therapy with X-ray alone. Using *Techniques A* or *C*, in 5 of 10 fields, hard nodes showed marked regression or disappearance. It could have been anticipated that the hard lesions would show very little response since, histologically, they show an essentially fibrotic change.

The results in the present report do not reflect the same degree of success with X-ray therapy of tuberculous lymphadenitis as do those of Lampe and associates. This may be due to the fact that the treatment was different from a technical standpoint: in total roentgens; the size and frequency of the individual dose; and in the fact that Lampe's technique called for the use of somewhat heavier filtration of rays (0.5 mm. of copper plus 1 mm. of aluminum), producing somewhat more penetrating rays. *Technique C* as described in the present report came the closest to approximating Lampe's technique in terms of technical factors and results.

In 1948, H. Bailey (8) reviewed the role of surgery in the treatment of cervical tuberculous lymphadenitis. Regarding X-ray treatment for this affliction, he states that the disadvantages of this form of treatment outweigh the good results, even if the latter occurred in a high percentage of cases. The disadvantages he mentions are the thickening and underlying fibrosis of the skin, the occasional X-ray burn, and the rare case of malignant skin disease following irradiation.

In the series of cases in the present study, deleterious effects of irradiation were not seen except for reddening and some induration of the surrounding skin in a few of the patients. None of the patients mentioned in the follow-up review showed any abnormality of the skin of the area other than some scarring in the patients with draining sinuses, when seen subsequently.

J. Burns Amberson (9) states that X-ray therapy of cervical tuberculous lymph-

adenitis is believed by many to be effective, particularly in cases of draining sinuses. He points out, however, that this therapy has contributed to breakdown and suppuration of the involved nodes. This would more likely occur during intense or protracted therapy. In the present series, this complication was not a serious feature. In the patient who received various types of treatment on several occasions for seven years and who was finally treated with intermittent, concentrated dosage, it was noted that the nodes became fluctuant and that the skin sloughed directly over the involved nodes. This occurred following the second dose. As irradiation was continued, however, the open area filled in with healthy granulation tissue and went on to healing.

The belief that the occurrence of miliary tuberculosis follows irradiation therapy of tuberculous lymphadenitis has been a great factor in the general lack of interest in this mode of treatment. The origin of this belief is not well documented in the literature. That such cases occurred is probably true, but whether they occurred merely in temporal relation to, rather than as a direct result of, X-ray therapy remains in question. A possible explanation may be found by considering the fact that peripheral tuberculous lymphadenitis is usually part of a subacute or chronic form of generalized hematogenous tuberculosis. Hence, progression of the disease in the lungs and elsewhere in the body may be expected in a certain number of cases, irrespective of whether X-ray irradiation of the lymph nodes has been performed.

It is of interest to note that the complication of miliary tuberculosis has not been well documented in the literature. None of the references except Lester (1) listed in the present report mention this complication, although other complications and disadvantages of X-ray irradiation in tuberculous lymphadenitis have been discussed.

Miliary tuberculosis did not occur in any of the cases reported in the present study. To be sure, if miliary tuberculosis did occur, it would be regarded as a more serious complication. The present writers agree that discretion must always be used in choosing the candidates for this type of therapy. Certainly, a patient in the toxic phase of the disease, or one who is greatly debilitated, should not receive X-ray irradiation.

It is doubtful that irradiation should be employed as a substitute for the use of antimicrobial agents, for tuberculous adenitis represents only one manifestation of the systemic tuberculous infection present at that time. It is believed, however, that the present results justify the conclusion that a trial of roentgen-ray therapy is indicated in conjunction with antimicrobial agents, and possibly as an adjunct to surgery, if irradiation does not preclude the latter method as reported by Lahey and associates (5). The exclusive use of X-ray therapy could be reserved for those few patients in whom antimicrobial agents have failed.

When discretion is used in choosing the candidates and the X-ray irradiation is administered judiciously and by qualified personnel, the frequency, severity, or permanence of the complications would not be of great enough extent to deter its use.

X-ray treatment given by the present writers in the future will be with the concentrated technique because it produces better results and economy of time.

SUMMARY

The results of X-ray treatment of 65 patients with tuberculous lymphadenitis representing 87 fields during an eight-year period are described. The techniques varied in some degree but all were of the intermittent, low-dosage variety, the average frequency of treatment being once a week. The over-all results showed a 35 per cent average healing rate when the nodes were hard, and a 63 per cent average healing rate when the nodes were fluctuant with or without drainage. No significant, permanent, deleterious effects of irradiation were noted.

SUMARIO

Roentgenoterapia de la Linfadenitis Tuberculosa Periférica

Descríbese el resultado de la roentgenoterapia de 65 enfermos, representando 87 campos, que tenían linfadenitis tuberculosa, durante un período de ocho años. Las técnicas variaron algo, pero todas fueron de la variedad intermitente de dosis baja, alcanzando los tratamientos una frecuencia media de una vez por semana. Los resultados globales revelaron un coeficiente medio de treinta y cinco por ciento de cicatrización cuando los ganglios eran duros y de sesenta y tres por ciento cuando los ganglios fluctuaban y/o drenaban. No se notaron efectos nocivos, importantes o permanentes, de la irradiación.

RESUME

Radiothérapie de l'adénite tuberculeuse périphérique

Présentation des résultats de la radiothérapie chez 65 malades, correspondant à 87 champs, atteints d'adénite tuberculeuse, enregistrés au cours d'une période de huit années. Les techniques variaient dans une certaine mesure, mais elles appartenaient toutes au type de la dose réduite et intermittente; la fréquence moyenne du traitement étant une fois par semaine. Les résultats d'ensemble montraient un taux moyen de cicatrisation de trente-cinq pour cent quand les ganglions étaient durs et un taux de cicatrisation moyen de soixante-trois pour cent quand les ganglions étaient fluctuants ou fistulisés, ou présentant ces deux aspects à la fois. Il n'a été observé aucun effet nocif permanent appréciable imputable à l'irradiation.

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THE EFFECT OF TESTOSTERONE ON PATIENTS WITH PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

Although tuberculosis is recognized as one of the most important etiologic agents in the production of Addison's disease, the clinician engaged in the treatment of tuberculous patients rarely encounters this entity. Nevertheless, in view of certain similarities in the symptoms of Addison's disease and pulmonary tuberculosis, reduced adrenocortical function has been suspected in patients with the latter disease.

The urinary 17-ketosteroids are metabolic end products of the adrenal cortical hormones in women and of the adrenal cortical and testicular hormones in men. Recently Bastenie and Kowalewski (1) and Rivoire and his associates (2) reported a significant decrease in the daily output of 17-ketosteroids in patients with active tuberculosis. This decreased excretion was correlated with the severity and duration of the disease. Bastenie and Kowalewski (1) noted no definite lesions in the adrenal glands of the autopsied cases. However, both groups of investigators were of the opinion that the toxicity (related to the stress syndrome) of the *chronic infection* caused a diminution in the excretion of the steroids. Rivoire and his co-workers attempted to restore 17-ketosteroid excretion to normal levels in patients with pulmonary tuberculosis by daily injections of large doses of testosterone (as high as 160 mg.). They stated that the injections were well tolerated and the output of 17-ketosteroids was restored to normal or near normal in many cases, with accompanying weight gain and euphoria.

The androgens are rather similar chemically to the hormones of the adrenal cortex and exhibit some of their metabolic features. Thus, the administration of testosterone causes retention of extracellular electrolytes and fluid. The site of action is probably on the renal tubules. In addition, the excretion of nitrogen, inorganic phosphorus, and potassium is decreased. The protein anabolic effect of testosterone merits emphasis on its possible therapeusis in a chronic debilitating disease such as tuberculosis.

An attempt to confirm, if possible, the therapeutic results of Rivoire and his co-workers (2) seemed worth while. The present study in a group of tuberculous patients was undertaken to observe: the effect of testosterone on the twenty-fourth-hour urinary excretion of the 17-ketosteroids; and the clinical effects of testosterone.

SELECTION OF PATIENTS AND METHOD OF STUDY

The patients selected for study had far advanced pulmonary tuberculosis and subnormal twenty-four-hour urinary excretion of 17-ketosteroids (8.0 mg. per

[1] From the B. S. Pollak Hospital for Chest Diseases, Jersey City, New Jersey.

twenty-four hours was used as the lower limit of normal). There was a total of 19 patients, all males, ranging in age from 23 to 67 years (average 46); 13 were white and 6 were Negro.

The patients were assigned to one of three categories:

Group A: Seven patients were treated with a testosterone propionate solution in sesame oil (Neo-Hombreol[1]) administered intramuscularly.

Group B: Six patients were treated with methyl testosterone orally (Neo-Hombreol (M)[2]). It is recognized that, although this androgen causes protein anabolic effects, it does not appear as 17-ketosteroid in the urine.

Group C: Six patients formed the control group and received placebo pills.

Clinical and laboratory observations were made prior to therapy, during therapy, and during a post-therapy control period. These included notations on the general condition, weight, blood pressure, prostatic size, mental reactions, and such determinations as the twenty-four-hour urinary excretion of 17-ketosteroids and the Robinson-Kepler-Power water test. The last-named test was done only *before* and *after* the completion of treatment. The usual roentgenographic and sputum studies were employed for the follow-up evaluation of the status of the pulmonary tuberculosis in each case.

RESULTS

In tables 1 and 2 may be seen the detailed information concerning the status and findings of the patients investigated. The following paragraphs summarize some of the pertinent data.

A grand total of 282 determinations of the twenty-four-hour urinary 17-ketosteroid excretion was done in the 19 patients; 38 before, 202 during, and 42 in the post-therapy observation period.

Pre-treatment determinations: All 38 pre-therapy determinations were below the 8 mg. level and ranged between 1.0 and 7.6 mg. per twenty-four-hour period. During placebo therapy and thereafter, the 6 patients in *Group C* excreted varied amounts, which ranged from 1.1 to 15.8 mg. per twenty-four hours, but the 6 average excretion values included only one distinctly increased 17-ketosteroid output.

Methyl testosterone orally: In *Group B*, in which the patients received methyl testosterone orally, the variation during and after therapy was 0.96 to 12.9 mg. per twenty-four hours. The impression gained of increased average excretion during the use of methyl testosterone is probably associated with clinical change in the disease, as the increased 17-ketosteroids are at least as apparent or more so during the ninety days of observation after cessation of methyl testosterone therapy. No 17-ketosteroids are to be expected as direct excretory products of methyl testosterone.

Testosterone propionate: During therapy with testosterone propionate the 7 patients of *Group A* excreted various quantities which ranged from 2.4 to 18.1 per twenty-four hours in 72 specimens. In each case there was a distinct sugges-

[1] Proprietary name for testosterone propionate supplied by Dr. Kenneth W. Thompson of Organon, Inc.

[2] Proprietary name for methyl testosterone supplied by Dr. Kenneth W. Thompson of Organon, Inc.

tion of increased average excretion during therapy. However, this amounted to an average increase of 1.9 mg. when the daily dose was 25 mg. and 2.1 mg. on 50 mg. daily, and 2.9 mg. per twenty-four hours when 75 mg. were injected. Return of 17-ketosteroid excretion to pre-treatment levels or below was noted in 2 of the 5 patients studied during the three months following therapy. While the administration of testosterone propionate did not produce a consistent pattern of normal excretion figures, it should be mentioned that this group showed more high normal peaks in contrast to the groups treated by placebo and methyl testosterone.

Robinson-Kepler-Power water test: Seventeen of the 19 patients had an abnormal Robinson-Kepler-Power water test at the beginning of the study. The test was repeated at the end of therapy in 10 of the 13 treated patients (one patient died and 2 others left the hospital against advice). The result was still abnormal in 9 of the 10 patients. All of the control patients had abnormal tests at the start and end of placebo therapy.

Clinical effects of testosterone: Several clinical effects of testosterone therapy should be mentioned. Among the 7 patients treated by injection, there were 2 (Cases 1 and 3) who developed edema with the 50 mg. dose. This disappeared after several days following withdrawal of the drug and the use of diuretics. Two patients (Cases 3 and 4) commented on considerably increased libido. Two patients (Cases 9 and 10) developed mild but painful gynecomastia with the dosage of 75 mg. daily and a drug rash appeared in one patient (Case 10). The eruption cleared with temporary cessation of therapy. No significant alterations from the pre-therapy status were noted in the blood pressure and size of the prostate.

Changes in body weight: The following comments, from the clinical point of view, are most important and pertain to weight changes (table 2). In *Group A*, treated with testosterone propionate, 6 patients (Case 5 excluded) showed a pre-therapy weight loss, compared with the maximum weight during hospitalization, and this ranged from 2 to 28 pounds (average, 12.6 pounds). It is significant that 3 of these patients (Cases 2, 3, and 4) lost weight while receiving double chemotherapy (streptomycin and para-aminosalicylic acid), which was started one to three days after admission. These drugs were then continued together with the androgen. Three patients (Cases 2, 3, and 4) gained 24, 39, and 56 pounds, respectively. The patients (Cases 1, 6, and 7) who had no antimicrobial therapy during the testosterone administration, gained 3, 5, and 11 pounds, respectively. The minimum treatment period with the hormone was 65 days; the maximum, 168; and the average, 113. Also of interest is the fact that in Cases 6 and 7 the patients developed a decline in weight following the discontinuance of testosterone. The weight gain in Case 1 was considered false and due to edema. One patient (Case 5) gained 13 pounds while receiving antimicrobial therapy and an additional 17 pounds when testosterone was added for a 96-day period prior to pulmonary resection.

In *Group B*, treated with methyl testosterone, the patients of Cases 9 and 12 experienced a pre-therapy weight loss of 8 and 12 pounds, compared with their

TABLE 1

ESTIMATIONS OF 24-HOUR URINARY 17-KETOSTEROIDS AND ROBINSON-KEPLER-POWER WATER TEST IN TREATED AND CONTROL CASES OF PULMONARY TUBERCULOSIS

		PRE-THERAPY				THERAPY						POST-THERAPY			
		17 K-S Mg. per 24 Hours						17 K-S Mg. per 24 Hours					17 K-S Mg. per 24 Hours		
	Case	Number of Tests	Range	Average	R-K-P Test	Daily Dose	Duration Days	Number of Tests	Range	Average	R-K-P Test	Observation Period Day	Number of Tests	Range	Average
	Group A—Treated with Testosterone Intramuscularly														
1	L. D., 47 years	2	4.9–5.7	5.3	Normal	25	60	8	3.4–13.0	7.8	Not done	57	0	*	
						50	5	1	2.6	2.6					
2	B. C., 26 years	2	5.8–6.6	6.2	Abnormal	25	56	8	2.4–14.0	6.6	Not done				
						50	56	4	2.5–17.6	8.8					
						75	9 (Signed release)	1	4.0	4.0					
3	H. S., 35 years	2	5.4–6.4	5.9	Abnormal	25	63	8	3.5–15.3	8.5	Abnormal	90	3	0.8–7.8	3.3
						50	42	3	2.7–7.6	4.4					
						75	56	4	4.1–16.8	9.3					
4	W. H., 41 years	2	2.8–5.0	3.9	Abnormal	25	56	8	2.8–11.3	6.0	Abnormal	67	1	16.0	16.0
						50	56	4	3.7–6.5	5.4					
						75	56	4	5.1–16.2	9.4					
5	A. S., 49 years	2	2.6–3.8	3.2	Abnormal	50	42	3	4.4–13.1	7.7	Abnormal	90	3	2.9–5.9	4.2
						75	56	3	4.0–5.5	4.8					
6	J. M., 58 years	2	3.6–4.0	3.8	Abnormal	50	42	3	10.7–18.1	13.6	Abnormal	90	3	2.1–5.0	3.8
						75	56	3	5.1–12.1	9.6					
7	R. P., 41 years	2	6.8–7.0	6.9	Abnormal	50	28	2	5.9–9.6	7.7	Abnormal	90	3	5.9–14.6	9.2
						75	56	5	5.9–16.2	10.2					

Group B—Treated with Testosterone Orally															
8	C. C., 23 years	2	1.7–3.9	2.8	Abnormal	25	49	6	2.2–8.5	4.9	Abnormal	90	3	2.9–7.3	5.2
						50	56	3	4.6–6.4	5.4					
						75	56	3	2.5–7.0	5.3					
9	S. S., 40 years	2	2.4–3.8	3.1	Normal	25	49	7	1.7–4.0	2.6	Normal	90	3	2.6–6.8	4.1
						50	49	4	0.96–3.9	2.7					
						75	49	3	2.4–2.7	2.5					
10	W. E., 40 years	2	1.6–2.8	2.2	Abnormal	25	49	7	1.4–10.6	4.5	Abnormal	90	3	2.2–12.9	5.7
						50	56	3	2.1–7.5	4.2					
						75	42	4	1.9–4.3	3.0					
11	B. H., 34 years	2	1.8–3.0	2.4	Abnormal	25	42	7	2.2–11.8	6.4	Not done				
						50	56	3	4.8–8.6	6.5					
						75	10 (Signed release)	1	4.6	4.6					
12	L. I., 65 years	2	1.0–2.4	1.7	Abnormal	50	42	2	5.9–6.8	6.3	Abnormal	90	3	3.8–8.6	5.9
						75	56	3	3.8–5.6	4.5					
13	J. J., 51 years	2	1.2–3.0	2.1	Abnormal	50	42	2	2.5–3.9	3.2	Abnormal	90	3	6.5–9.2	7.5
						75	56	3	2.2–3.1	2.7					
Group C—Treated with Placebo															
14	S. D., 67 years	2	1.8–2.8	2.3	Abnormal		168	13	1.6–8.8	4.4	Abnormal	90	3	1.4–10.5	4.7
15	J. M., 50 years	2	2.4–4.8	3.6	Abnormal		168	10	1.3–4.5	2.6	Abnormal	90	3	1.9–5.0	3.0
16	W. Mc., 50 years	2	6.8–7.6	7.2	Abnormal		168	14	1.7–11.5	4.1	Abnormal	90	2	3.8–4.1	3.8
17	D. M., 52 years	2	5.4–6.2	5.8	Abnormal		168	14	2.2–12.9	6.9	Abnormal	90	3	3.1–9.4	5.4
18	R. Mc., 65 years	2	1.6–3.0	2.3	Abnormal		168	13	1.1–4.9	3.1	Abnormal	90	3	1.9–4.7	3.6
19	P. L., 49 years	2	5.4–7.0	6.2	Abnormal		98	5	5.9–15.8	11.9	Abnormal	10 (Signed release)			

* Treatment ended because of very poor condition on July 27, 1951; patient died September 24, 1951.

maximum weight. Both received antimicrobial therapy before the use of the oral androgen product. When the latter was stopped after 147 and 96 days, one of the patients (Case 9) had not gained any weight, while the other (Case 12) had gained 3 pounds. Prior to methyl testosterone medication, the remaining 4 patients had gained from 2 to 30 pounds and had received antimicrobial therapy. At the end of androgen therapy, the patients of Cases 10, 11, and 13 had gained 9 to 23 pounds, while one patient (Case 8) lost weight (developed superimposed miliary tuberculosis). The minimum period of hormone administration was 98 days; the maximum, 161; and the average, 126.

In *Group C*, 4 patients gained 1 to 11.5 pounds during the use of the placebo; 3 of the 4 also received antimicrobial therapy during this interval. The minimum duration of placebo treatment was 98 days; the maximum, 168; and the average, 156.

In summarizing the weight changes, it is seen that: in the group treated with testosterone propionate, 6 patients (85 per cent) gained an average of 25.1 pounds; in the methyl testosterone group, 4 patients (66 per cent) had gained

TABLE 2

TOTAL NUMBER OF URINARY ESTIMATIONS OF 17-KETOSTEROIDS AND AVERAGE VALUES (MG. PER 24 HOURS) IN TREATED AND CONTROL GROUPS

	PRE-THERAPY		DURING THERAPY		POST-THERAPY	
	Number of Tests	Average Value	Number of Tests	Average Value	Number of Tests	Average Value
Group A	14	5.0	72	7.5	13	7.3
Group B	12	2.4	61	4.3	15	5.7
Group C	12	4.6	69	5.5	14	4.1

an average of 14.5 pounds; in the control group, 4 patients (66 per cent) had gained an average of 5.3 pounds. In addition, a sense of increased well-being (moderate euphoria) was observed in 5 patients (Cases 2, 3, 4, 11, and 13) during testosterone therapy, but this feeling definitely receded in the 3 patients who were observed in the post-therapy period. How much this attitude should be attributed to a psychological effect of the therapeutic regimen would be difficult to say.

DISCUSSION

Studies to evaluate adrenocortical function in pulmonary tuberculosis have been few. Some investigators have postulated the presence of disturbed function in some cases on the basis of various estimations, such as the "protective-ferment reaction" (3), the hyaluronidase-fluorescence skin test, the eosinophile test of Thorn (4), the Robinson-Kepler-Power water test (5), the sodium chloride deprivation test (6), and the urinary excretion of 17-ketosteroids (1, 2). There appears to be no generally accepted test of adrenal functional capacity in the human. In the present study, two tests to "assay" adrenocortical function were

used: the Robinson-Kepler-Power water test and the twenty-four-hour urinary excretion of 17-ketosteroids.

The role of the adrenal cortex in electrolyte and water metabolism was one of the first to be emphasized. Adrenal insufficiency is characterized by the renal loss of sodium salts and deficiency in water diuresis. Thorn and his co-workers (6) made a significant contribution in their study of electrolyte changes in pulmonary tuberculosis with relation to adrenocortical function and noted these points of difference from patients with Addison's disease. In the group with pulmonary tuberculosis, the following changes were noted:

> A normal serum concentration of sodium and potassium was present, accompanied by a decrease in serum chloride which paralleled the reduction in the vital capacity. This reduction was attributed largely to an accumulation of carbon dioxide as a result of pulmonary changes. Nevertheless, the drop in the serum chloride was not accompanied by an equivalent reduction in the serum sodium, which always occurs in Addison's disease.
>
> A normal or increased value (in extensive disease) of serum bicarbonate concentration was present, whereas in Addison's disease it is either normal or reduced.
>
> The ability to maintain a normal total blood volume was preserved.
>
> The ability to tolerate sodium chloride deprivation (with supplementary intake of potassium) without exhibiting the symptoms of adrenal cortical insufficiency such as weight loss, fall in blood pressure, and urinary loss of chloride and sodium was also present.

Apparently serum sodium may be significantly reduced in patients with pulmonary tuberculosis, with or without eventual histopathologic evidence of adrenal cortical disease (7). This seems to be at variance with the above report (6).

The sodium chloride deprivation test was not employed in the present study but deficiency in water diuresis was measured by the Robinson-Kepler-Power test. In the absence of adequate amounts of cortical hormone, ingested water is not excreted at a normal rate. In most instances, adrenal cortical insufficiency can be excluded if water excretion is normal. However, if water excretion is subnormal, the test does not necessarily indicate adrenal inadequacy, since other conditions such as nephritis, acidosis, hepatic cirrhosis, and poor intestinal absorption may give a similar result.

The 17-ketosteroids consist of a group of compounds which have in common a ketone group at position 17 of the steroid nucleus. Because of diurnal variations in their excretion, the determination should be made on a twenty-four-hour rather than a single specimen of urine. According to the method (8) used in the present study, the range of normal values for males is 8 to 20 mg. per twenty-four hours and 5 to 15 mg. for females. Thus, in interpreting the significance of 17-ketosteroid excretion, it is important to keep in mind that the rate of excretion is not constant during a twenty-four-hour period; it varies from day to day and covers a rather wide normal range. Moreover, there are quantitative variations, not only with sex, but also with age. The general pattern is that excretion is lowest in children, increases in adolescence, reaches its peak usually

between the ages of 20 to 40 years, and declines slowly thereafter. Thus, by the seventh decade, the average amount is only approximately one-half that excreted by males in the third and fourth decades. This is usually attributed to decreased testicular and adrenal cortical production of steroid hormones. In this regard it should be mentioned that the average age of the testosterone-treated patients was 32.3 years and that of the control group 55 years.

Because of the rather wide quantitative fluctuations which may be misleading in the occasionally performed estimations of urinary 17-ketosteroids, it is important that an opinion be based on frequent *serial* determinations to smooth out the curve of variations. While a consistently normal rate of excretion of 17-ketosteroids would tend to exclude the presence of adrenal cortical insufficiency, caution is necessary in assuming that a lowered excretion is evidence of a decrease in function. This may be seen in other conditions, particularly diabetes, hepatic disease (acute or chronic), and starvation. Moreover, there is always the definite possibility that, in disease or advancing age, there may be some change in the metabolic disposal (9) or utilization of the steroid hormones or their precursors.

Since the 17-ketosteroid excretions of all patients in the pre-treatment period were of amounts considered low normal or subnormal for adult females, it is believed that these patients may have had reduced function of both testis and adrenal tissue.

Dorfman and Hamilton (10) studied the absorption and excretion of testosterone propionate in man. They found that the crystalline product was promptly absorbed from the gastrointestinal tract and excreted through the kidneys in large amounts with only transient retention in the body. After the daily intramuscular injections of 20 mg. of testosterone propionate in peanut oil for thirty successive days, the level of androgen excretion in a patient with deficient testicular function was raised to the normal range. However, the androgen activity of the urine was approximately 6 per cent of the amount given, indicating that a large amount of the injected testosterone was either destroyed or changed chemically. The wide and unexplained variation in the proportion of injected testosterone propionate which appears as 17-ketosteroid from day to day in a normal male has been commented upon by others (13). In view of this, it is not too surprising to learn from the present study with far advanced cases of tuberculosis (in which the factor of chronic stress and chronic infection is apparent) that the intramuscular use of testosterone propionate in daily doses varying from 25 to 75 mg. had no striking effect on the urinary 17-ketosteroid excretion. The apparent increased 17-ketosteroid excretion with methyl testosterone was merely evidence of fluctuations in daily values of the same magnitude found in the placebo group (C).

Selye (11) has popularized the "alarm reaction" and the stimulating effect of stress on adrenal cortical activity. While it might be assumed, therefore, that the excretion of 17-ketosteroids is increased during stress, in actual determinations it has been found that, in previously normal persons subjected to medical or surgical injury, the general excretory pattern consists of a temporary increase in urinary 17-ketosteroids for one to three days, followed by a drop below

control values and a gradual return to normal as complete cure is obtained. Another interesting point in the stress situation is the presence of some evidence (12) of increased cortical activity even during the period of decreased 17-ketosteroid excretion. This evidence is found in the increased excretion of glycogenic corticosteroids and physiologic changes attributed to these steroids.

Writers on adrenocortical function in pulmonary tuberculosis are in agreement that any impairment of such function, if it occurs, is apt to be encountered most commonly in far advanced active disease of considerable duration. In view of some of the above considerations, the question arises whether tests in these patients actually mirror this function accurately—especially when impaired intestinal absorption, fatty liver, and amyloidosis (hepatic and renal), which notoriously are more prevalent in the sicker tuberculous patient, may modify the results of some of the tests. The present writers definitely believe that this cautious attitude is applicable to the two tests used in the present study, namely, the Robinson-Kepler-Power test and the twenty-four-hour urinary estimation of the 17-ketosteroids. Furthermore, in the writers' experience, there is little anatomic evidence of unusual changes in the adrenal cortex in cases of pulmonary tuberculosis on autopsy examination.

It has been amply demonstrated that testosterone propionate has a protein anabolic effect in a number of conditions (14–17), with retention of nitrogen, phosphorus, and potassium, favoring the synthesis of protoplasm. Methyl testosterone also promotes metabolism of protein but, unlike the propionate, it produces marked creatinuria. For this reason apparently, the former, for example, aggravates the clinical picture of thyrotoxicosis (18), whereas the propionate is helpful and decreases the hypercreatinuria which is present in this disease. Newman and his associates (19), in their study of a few cases of pulmonary tuberculosis, observed that the intramuscular use of testosterone produced a markedly positive nitrogen balance when given alone or with a protein hydrolysate (amigen) intravenously. The writers have the definite impression from the present limited experiment that testosterone propionate proves itself worthy of trial as a therapeutic tool in tuberculous patients in whom a weight gain is desirable. This can be of particular value in the pre- and postoperative periods of thoracic surgery. A dose of 50 mg. two or three times weekly is probably adequate and would decrease the incidence of untoward reactions. Daily administration was used in the present study as the effect on the twenty-four-hour urinary excretion of 17-ketosteroids was also under study. The accompanying subjective sense of well-being and alertness which may be present in some patients is a favorable psychological by-product of the testosterone propionate therapy.

It is important to watch for the possible development of edema, which will lead to a false weight gain. If there is no contraindication, the employment of a salt-poor diet will minimize this complication. In many patients, the use of antimicrobial therapy leads to a substantial increase in weight. It appears that the additional administration of testosterone propionate may accelerate this tendency. The untoward effects noted were not serious and cleared rather

promptly with cessation of therapy. The presence of carcinoma of the prostate should be excluded prior to the use of androgen therapy, and in females the total monthly dose should probably not be in excess of 300 mg. to avoid the possibility of masculinizing effects. The occurrence of edema and possibility of other side effects caused the present writers to use smaller doses than in the studies of Rivoire and his associates (2).

SUMMARY

The water diuresis test and excretion of 17-ketosteroids were used as criteria of reduced adrenal cortical function in selecting 19 male patients with far advanced pulmonary tuberculosis. Limitations of the probable validity of these tests in interpreting adrenocortical function in these cases are mentioned. Six patients were treated with placebos; 7 with testosterone propionate, 25 to 75 mg. intramuscularly daily; and 6 with methyl testosterone, 25 to 75 mg. orally daily. No significant changes were observed in water diuresis. The excretion of 17-ketosteroids was probably increased, though only slightly, in the group which received testosterone propionate. The doses of androgens were adequate to cause several instances of edema and of gynecomastia, which were all reversible in type.

Weight gains occurred in all groups. Nevertheless, the weight gains were far more striking in the patients who received testosterone propionate, and were of slight degree in those treated with placebos. Apparently, protein anabolic effects can be obtained with doses too small to cause edema, gynecomastia, or significantly increased excretion of 17-ketosteroids.

SUMARIO

El Efecto de la Testosterona sobre los Enfermos con Tuberculosis Pulmonar

La prueba de la diuresis hídrica y la excreción de 17-quetosteroides fueron utilizadas como normas para determinar la existencia de hipofunción corticosuprarrenal en la selección de 19 enfermos varones con tuberculosis pulmonar muy avanzada. Menciónanse las limitaciones de la probable validez de dichas pruebas en la interpretación de la función corticosuprarrenal en esos casos. A 6 se les trató con placebos; a 7 con propionato de testosterona, 25 a 75 mg. intramuscularmente a diario; y a 6 con metiltestosterona, 25 a 75 mg. diarios oralmente. No se observaron alteraciones de importancia en la diuresis hídrica. La excreción de 17-quetosteroides aumentó probablemente, aunque sólo en forma ligera, en el grupo que recibió propionato de testosterona. Las dosis de andrógenos fueron suficientes para producir varios casos de edema y de ginecomastia, de forma reversible.

Hubo aumento de peso en todos los grupos, pero mucho más notable en los sujetos que recibieron propionato de testosterona, y de poca cuantía en los tratados con placebos. Aparentemente, pueden obtenerse efectos anabólicos sobre las proteínas con dosis insuficientes para provocar edema, ginecomastia o hiperexcreción significativa de 17-quetosteroides.

RESUME

L'action de la testostérone chez les malades atteints de tuberculose pulmonaire

L'épreuve de la diurèse provoquée et l'excrétion des 17-cétostéroïdes ont été utilisées comme critère de la réduction de la fonction cortico-surrénale dans un groupe de 19 hommes atteints de tuberculose pulmonaire avancée. Dans ces cas, les auteurs indiquent les limita-

tions de la valeur possible des tests pour l'interprétation de la fonction cortico-surrénale. Six des sujets ont été traités avec des "placebos" (médicaments fictifs); sept avec le propionate de testostérone intramusculaire à la dose de 25 à 75 mg. par jour; 6 avec la méthyltestostérone orale à la dose de 25 à 75 mg. par jour. Aucune modification significative de l'épreuve de diurèse provoquée n'a été observée. L'excrétion des 17-cétostéroïdes était peut-être légèrement augmentée dans le groupe recevant le propionate de testostérone. Les doses d'androgène ont été suffisantes pour provoquer plusieurs cas d'oedème et de gynécomastie de type réversible.

Des gains de poids se sont produits dans tous les groupes, mais ils out été beaucoup plus marqués chez les malades traités par le propionate de testostérone, ils étaient minimes chez ceux qui recevaient les placebos. Il semble qu'une action anabolique protéique puisse être assurée avec des doses trop faibles pour provoquer l'oedème, la gynécomastie ou une augmentation significative de l'excrétion des 17 cétostéroïdes.

Acknowledgment

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THE DETERMINATIONS OF BLOOD FLOW THROUGH NONVENTILATED PORTIONS OF THE NORMAL AND DISEASED LUNG[1, 2]

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INTRODUCTION

In the normal lung, nearly all of the blood from the pulmonary artery flows through capillaries which traverse functioning alveoli. In various pulmonary diseases, a portion of the blood from the pulmonary artery may flow through nonventilated lung tissue. When this blood enters a pulmonary vein, it has the same oxygen content as blood from the pulmonary artery. In essence, this admixture may be considered as an arteriovenous shunt. The magnitude of the shunt depends upon the extent and character of the disease of the lung and the blood flow through the diseased area.

A method has been devised by Berggren (1) for measuring shunts between the pulmonary artery and pulmonary vein. The patient breathes 100 per cent oxygen for a sufficient period of time to replace nitrogen in the alveoli with the oxygen. A sample of blood from a systemic artery and a sample of alveolar air are obtained simultaneously. The oxygen tension in each of these samples is determined by appropriate means. The difference between the oxygen tension in the alveolar air and in the arterial blood represents the approximate magnitude of the shunt through the nonventilated lung. Determinations are made while the patient is breathing 100 per cent oxygen in order to minimize the effect of impaired diffusion of oxygen through the alveolar membrane and unequal alveolar ventilation on the determination of the magnitude of the shunt.

The purpose of the present study was to apply this method to patients with various types of pulmonary disease in order to obtain information regarding the circulation in diseased areas of the lung. Particular emphasis was placed on a group of patients with unilateral pulmonary disease in whom bronchospirometry demonstrated a decreased oxygen uptake in the involved lung.

METHOD

The difference in oxygen tension between the alveolar air and arterial blood was measured in 11 normal men, 11 patients with advanced bilateral pulmonary tuberculosis, 14 patients with bronchogenic carcinoma, 8 patients convalescing from pneumonia, 10 patients with pleural effusion, 12 patients with pulmonary

[1] From the Veterans Administration Hospital; and Department of Medicine, University of Minnesota, Minneapolis, Minnesota.

[2] Presented before the Central Society for Clinical Research, Chicago, Illinois, on November 2-3, 1951.

emphysema, 8 patients with chronic cardiac disease, and 10 patients with hepatic cirrhosis (table 1). Twenty additional patients were studied in whom bronchospirometry had demonstrated impaired function of one lung (table 2). The oxygen tensions of the alveolar air and arterial blood were measured after the patients had breathed 100 per cent oxygen for twenty minutes.

The oxygen tension in the blood was measured directly with the polarograph (1, 2, 3) (figure 1). A femoral artery puncture was made with a Cournand needle. After a steady flow of arterial blood had been established through the needle, a heated syringe containing 0.1 cc. of heparin was attached. The pressure of the blood from the artery was allowed to force the plunger of the syringe to the 6 cc. level. The syringe was immediately placed in a high-speed angle centrifuge, spun for exactly two minutes, and stopped within one minute. It was essential to separate the cells from the plasma rapidly because of the consumption of

TABLE 1

THE MEANS AND STANDARD DEVIATIONS OF THE OXYGEN SATURATION OF THE HEMOGLOBIN AND MAGNITUDE OF THE ARTERIOVENOUS SHUNT IN THE PULMONARY DISEASES STUDIED

DISEASE	NUMBER OF PATIENTS	OXYGEN SATURATION OF HEMOGLOBIN OF ARTERIAL BLOOD (Per Cent)	ALVEOLAR ARTERIAL OXYGEN TENSION DIFFERENCE (Mm. of Mercury)	MAGNITUDE OF SHUNT (Per Cent of Total Pulmonary Blood Flow)
Pulmonary tuberculosis, far advanced	11	94.6 ± 1.8*	53 ± 37*	3.5 ± 2.3*
Pneumonia, resolving	8	91.2 ± 5.5	99 ± 32	6.5 ± 2.0
Bronchogenic carcinoma	14	90.3 ± 4.7	110 ± 94	6.9 ± 4.7
Pulmonary fibrosis	6	91.2 ± 6.0	80 ± 74	5.0 ± 4.5
Pleural effusion or empyema	10	90.8 ± 3.5	116 ± 51	7.7 ± 3.0
Pulmonary emphysema	12	91.8 ± 6.9	69 ± 50	5.4 ± 3.6
Congestive heart failure	8	94.4 ± 3.8	63 ± 55	4.1 ± 2.9
Cirrhosis of the liver	10	94.4 ± 3.9	88 ± 47	5.8 ± 2.9
Normal persons	11		16 ± 11.0	1.0 ± 0.8

* All values represent mean and standard deviation.

oxygen by the cells. The plasma was ejected into a 2 cc. cuvette. The cuvette had a narrow neck 1.5 cm. in length and an internal bore of 1.5 mm. to prevent exchange of oxygen between the plasma and the atmosphere.

The polarograph[3] was set so that the oxygen in the plasma would be reduced at an electromotive force of 0.5 volt. The diffusion current of oxygen at this voltage is a linear function of the tension of the gas in solution in the plasma (1, 3). A factor for converting polarographic readings to oxygen tension was obtained by equilibrating blood in tonometers at different oxygen tensions and obtaining polarographic readings (figure 2). The blood from the tonometer was centrifuged for exactly the same period of time as blood from the patients so that the consumption of oxygen by the cells would be comparable. In performing the procedure it is important that the syringes containing heparin, the cuvette, the centrifuge, the water bath, and the mercury in the dropping electrode reservoir be kept at a temperature of 37.5°C.

[3] A polarograph manufactured by Sargent Co., Chicago, Illinois, was used.

TABLE 2

THE MEAN AND STANDARD DEVIATION OF THE OXYGEN SATURATION OF HEMOGLOBIN, OXYGEN UPTAKE OF THE RIGHT AND LEFT LUNGS, AND MAGNITUDE OF THE SHUNT IN THE PATIENTS STUDIED WITH BRONCHOSPIROMETRY

SUBJECT	AGE	OXYGEN SATURATION OF HEMOGLOBIN OF ARTERIAL BLOOD*	PULMONARY OXYGEN UPTAKE			ALVEOLAR ARTERIAL OXYGEN TENSION DIFFERENCE	MAGNITUDE OF SHUNT	DIAGNOSIS
			Right Lung	Left Lung	Total			
		(Per Cent)	(Per Cent)		(cc./ Min.)	(Mm. of Mercury)	(Per Cent of Total Pulmonary Blood Flow)	
1	41	97.1	70.1	29.9	375	52.0	3.6	Bilateral pulmonary tuberculosis with left pneumothorax, re-expanded
2	61	94.4	32.5	67.5	501	17.5	1.2	Bilateral pulmonary tuberculosis, with thoracoplasty, right lung
3	59	95.9	5.4	94.6	332	96.3	6.3	Pulmonary tuberculosis, right lung, far advanced
4	55	97.2	38.5	61.5	390	59.2	4.0	Pulmonary tuberculosis, right lung, far advanced
5	30	92.2	67.9	32.1	280	47.0	3.2	Pulmonary tuberculosis, left decortication
6	24	95.5	27.8	72.2	360	100.5	6.6	Disseminated pulmonary tuberculosis, far advanced with right pleural effusion
7	32	91.5	88.7	11.3	301	91.6	6.1	Pulmonary tuberculosis, left lung, with tuberculous empyema
8	35	97.9	41.4	58.9	360	36.1	2.5	Bilateral pulmonary tuberculosis, with right pneumothorax
9	25	92.1	84.2	15.8	475	125.7	8.1	Pulmonary tuberculosis, left thoracoplasty
10	41	93.2	67.9	32.1	280	47.0	3.2	Bilateral pulmonary tuberculosis, far advanced
11	35	93.4	1.6	98.4	317	65.1	4.5	Pulmonary tuberculosis with right thoracoplasty
12	31	94.4	33.4	66.6	413	55.0	3.5	Bilateral pulmonary tuberculosis, with first right rib resection
13	54	90.8	89.0	11.0	272	20.5	1.4	Pulmonary tuberculosis, left lung, with thickened pleura
14	31	91.9	60.0	40.0	500	70.4	4.7	Bilateral pulmonary tuberculosis, with pneumoperitoneum
15	26	94.9	56.0	44.0	416	41.3	2.7	Bilateral pulmonary tuberculosis, with pneumothorax re-expanded on the right
16	27	94.4	87.5	12.5	400	122.5	7.9	Bilateral pulmonary tuberculosis, far advanced
17	40	91.7	74.5	25.5	470	148.1	9.5	Pulmonary tuberculosis with left pleural effusion
18	25	92.6	75.0	25.0	300	183.2	11.4	Tuberculous empyema, left pleural cavity
19	33	93.5	97.5	2.5	460	77.2	5.01	Bronchiectasis, nonfunctioning left lung
20	57	94.6	37.1	62.9	318	25.7	1.8	Tumor, right bronchus, with destruction of right lung
Mean		93.9600				73.095	4.42	
Standard deviation		±2.03				±44.10	±2.76	

* Corrected for dissolved oxygen, breathing room air.

While the blood was being drawn from the femoral artery, duplicate alveolar gas samples were collected by the method of Haldane and Priestley (4). The percentage of oxygen in the gas samples was measured by the method of Van Slyke and Sendroy (5). The oxygen

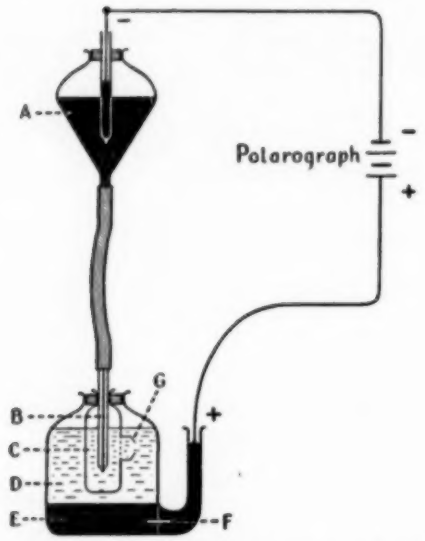

FIG. 1. The falling drop electrode and electrolysis cell.

A. Mercury reservoir
B. Dropping mercury electrode
C. Cuvette for plasma
D. Saturated potassium chloride solution
E. Mercury anode
F. Platinum wire bridge
G. Sintered glass agar bridge

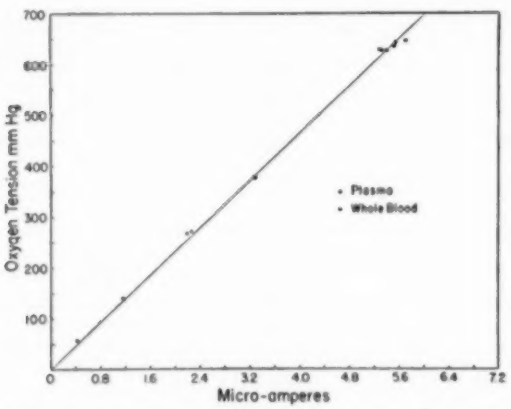

FIG. 2. The points on the chart represent the polarographic readings for whole blood and plasma equilibrated with a known partial pressure of oxygen. The relationship between oxygen tension and the polarographic diffusion current is linear.

tension in the alveoli was calculated from the known barometric pressure less the vapor pressure of water at 37.5°C.

The following formula was used to calculate the admixture of venous blood with blood from ventilated alveoli (6):

$$\text{Magnitude of the shunt} = 100 - \left(\frac{y}{y + G} \times 100\right)$$

y = arteriovenous oxygen difference

In the present study, y was taken as 4.29 volumes per 100 cc. This was based on the mean arteriovenous oxygen difference in 29 patients measured by right heart catheterization and femoral arterial punctures. G equals the difference between oxygen tension in the alveolar air and arterial blood multiplied by the Sendroy factor (7) for whole blood at 37.5°C., which is 0.003026. This factor was used to convert oxygen tension in mm. of mercury to volumes per 100 cc. of oxygen in solution.

RESULTS

The mean values for the difference in oxygen tension between alveolar air and arterial blood and for the magnitude of the shunt are listed in table 1. The data include studies on patients with pulmonary tuberculosis, those convalescing from pneumonia, those with bronchogenic carcinoma, pulmonary fibrosis, pleural effusion or empyema, pulmonary emphysema, congestive heart failure, cirrhosis of the liver, and normal persons. The oxygen saturation of the hemoglobin of the arterial blood in 11 normal persons was assumed to be within the normal range. The mean alveolar arterial oxygen tension difference was 16 mm. of mercury, and the mean magnitude of the pulmonary shunt was 1.0 per cent of the total pulmonary blood flow.

The level of the mean oxygen saturation of the hemoglobin of the arterial blood was significantly decreased in all groups of patients. The patients with the lowest hemoglobin saturation had the largest shunt and the greatest alveolar arterial oxygen tension difference. The magnitude of the pulmonary shunts in all groups (table 1) ranged from three to seven times the normal range. The patients with cirrhosis of the liver had no demonstrable pulmonary disease. Nevertheless, the mean oxygen saturation of the hemoglobin of the arterial blood was 94 per cent; the mean alveolar arterial oxygen tension difference was 88.0 mm. of mercury, which was more than five times the difference in normal persons, and the magnitude of the mean shunt was more than five times that of normal persons.

Similar studies on a group of patients on whom bronchospirometry was also performed are detailed in table 2. All of these patients had severe pulmonary disease and many of them had been treated for tuberculosis with collapse therapy. There was impairment of ventilation of the involved lung in all cases.

The oxygen uptake of the involved lung varied from 1.6 to 44 per cent of the total oxygen uptake for both lungs. The mean difference between the oxygen tension in the alveolar air and the arterial blood was 73.1 mm. of mercury, and the mean value for venous admixture was 4.4 per cent. In patients with severe chronic pulmonary fibrosis with tuberculosis, the oxygen uptake of the diseased lungs tended to be greatly decreased, as shown by bronchospirometry (table 2). The magnitude of the pulmonary shunt in the most severely diseased lungs with

a low oxygen uptake was within the range of the normal persons. This is illustrated in table 2, Cases 2, 13, and 20.

The following cases are described to illustrate the findings in the group of patients studied with bronchospirometry:

A roentgenogram of the chest of a 53-year-old patient (No. 13, table 2), with marked thickening of the pleura on the left from an old tuberculous effusion, is illustrated in figure 3. The patient was known to have had active pulmonary tuberculosis previously. The bronchospirometric data are shown below the roentgenogram. The oxygen uptake of the left lung was 11.0 per cent and that of the right lung was 89.0 per cent of the total. The alveolar arterial oxygen tension difference was 20.5 mm. of mercury, and the shunt was 1.4 per cent.

In figure 4 is shown the roentgenogram of the chest of patient No. 7 (table 2). He was a 32-year-old man who was found to have active pulmonary tuberculosis in 1948. During January, 1949, a left pneumothorax was initiated followed by a pneumonolysis. He subsequently developed tuberculous empyema. Cultures of the pleural fluid were found to contain tubercle bacilli. The bronchospirometric data and the venous admixture are listed below the roentgenogram. The oxygen uptake of the left lung was 11.3 per cent and that of the right lung was 88.7 per cent of the total; the alveolar arterial oxygen tension difference was 91.6 mm. of mercury. The venous admixture of 6.1 per cent is larger than it was in the previous patient, possibly because the disease is of more recent origin.

The roentgenogram of the chest of patient No. 11 (table 2) is illustrated in figure 5. This patient was found to have active pulmonary tuberculosis in 1943. In 1948 he had an extensive thoracoplasty. The sputum continued to contain tubercle bacilli. The oxygen uptake of the left lung was 1.6 per cent and that of the right was 98.4 per cent of the total. Alveolar arterial oxygen tension difference was 65.1 mm. of mercury, and the venous admixture or shunt was 4.5 per cent. A right pneumonectomy was done on March 15, 1951. The histologic examination of the removed lung showed caseous necrosis and extensive fibrosis of the parenchyma and pleura. The alveolar septa were thickened, fibrosed, and infiltrated with inflammatory cells.

DISCUSSION

According to Berggren (1), the method utilized in the present study measures blood flow through the nonventilated lung. While this appears to be true in general, several possible errors must be considered.

Marked discrepancy in alveolar ventilation may affect the results of the method. This is particularly true in pulmonary emphysema, where it is known that very long periods of oxygen breathing are required to replace the nitrogen in poorly ventilated alveoli and bullae. In order to minimize this error, a period of twenty minutes of oxygen breathing was chosen. In spite of this, a difference in oxygen tension of 69 mm. of mercury between alveolar air and arterial blood was present in the group of patients with emphysema. It seems likely that this was related to failure to replace the nitrogen in all areas of the lung. This error is probably not of great importance in the other diseases studied. The distinction between marked impairment of diffusion through alveolar walls and a shunt through the nonventilated lung is, to a certain extent, arbitrary. During the

FIG. 3 FIG. 4 FIG. 5

FIG. 3. The roentgenogram of patient No. 13, demonstrating markedly thickened pleura on the left. Bronchospirometry revealed the oxygen uptake of the right lung to be 242 cc. per minute and of the left lung to be 30 cc. per minute. The venous admixture was 1.4 per cent.

FIG. 4. The roentgenogram of patient No. 7, demonstrating tuberculous empyema on the left. Bronchospirometry revealed the oxygen uptake of the right lung to be 267 cc. per minute and of the left lung to be 34 cc. per minute. The venous admixture was 6.1 per cent.

FIG. 5. The roentgenogram of patient No. 11, demonstrating a thoracoplasty for therapy for tuberculosis on the right. Bronchospirometry revealed the oxygen uptake of the right lung to be 5 cc. per minute and of the left lung to be 317 cc. per minute. The venous admixture was 4.5 per cent.

breathing of 100 per cent oxygen, diffusion may occur through alveolar walls which allow little or no diffusion of oxygen when breathing air. Finally, admixture of blood from the bronchial veins into the pulmonary circulation is measured as a part of the blood flow through the nonventilated lung.

In spite of these potential errors, it appears that the method of Berggren gives an excellent estimate of blood flow through the nonaerated lung. The results obtained cannot be compared with the method of Lilienthal, Riley, Proemmel, and Franke (8). Their method for determination of venous admixture consists of measurement of the difference between the oxygen tension in alveolar air and arterial blood while breathing room air as compared with that while breathing gas with a low oxygen content. The value obtained for venous admixture includes blood which perfuses poorly ventilated alveoli.

The magnitude of venous shunts found in normal individuals in the present study is comparable to that found by Berggren (1) and somewhat less than that found by Morgan and Nahas (9). This small venous shunt in normal persons includes blood from the bronchial veins draining into the pulmonary veins through anastomoses. It thus appears that, in the normal lung, practically all of the blood from the pulmonary artery is distributed to ventilated alveoli.

The patients with pulmonary disease in the present study demonstrated a remarkably small circulation of blood through the nonventilated lung. This finding was not unexpected in pulmonary tuberculosis. It is well known that the lesions in this disease are relatively avascular. Pathologic examination of the lungs of patients with this disease have shown marked narrowing or obliteration of the small pulmonary arteries in areas involved by the disease (10). Injection of the pulmonary vascular system with a radiopaque substance has demonstrated marked diminution in the vascularity of areas of the lung involved by pulmonary tuberculosis (11). The relatively small venous shunt in patients convalescing from pneumonia can also be correlated with anatomic studies which illustrate a marked diminution in vascularity of the involved area of the lung (12).

In the group of patients with bronchogenic carcinoma and the group of patients with pleural effusion or empyema there was extensive compression or atelectasis of the lung. In spite of the compression or atelectasis, the magnitude of blood flow through the nonventilated lung was not large. This may be related, in part, to mechanical compression of the vessels in the atelectatic area, with resultant reduction in blood flow. There is recent evidence, however, which suggests that the diminution of blood flow may be related partly to the local hypoxia in the atelectatic area (2, 13, 14, 15). It has been shown that, if one lung is ventilated with nitrogen and one lung with oxygen, there is a marked diminution of blood flow to the lung breathing nitrogen.

The patients studied with bronchospirometry had predominantly unilateral disease. In most instances there was pleural disease or collapse therapy on the involved side. Bronchospirometry revealed a decrease in the oxygen uptake of the involved lung as well as impairment of ventilatory function. The amount of venous shunt was small and there appeared to be no correlation between the size of the shunt and the degree of impairment of oxygen uptake of the involved

lung ($r = 0.2$, $p > 0.1$). The reduction in oxygen uptake of a lung involved by pleural disease or subject to collapse therapy has usually been assumed to be the result of a diminution in blood flow (16). The results of the present study substantiate this idea, for a large venous shunt would be evident if a reduction in oxygen uptake of a lung were not accompanied by a corresponding reduction in blood flow.

The patients with cirrhosis of the liver were studied because of the occasional occurrence of a decrease in oxygen saturation of the hemoglobin of the arterial blood in this disease (17) and because of the possibility of the presence of pulmonary vascular abnormalities similar to the spider nevus of the skin. The small shunt found is difficult to interpret. It could be related to small areas of atelectasis rather than to any specific vascular abnormality.

Lundsgaard and Van Slyke (18), in their discussion of cyanosis, listed the following factors as contributory to cyanosis:

1) the total hemoglobin content of the blood;
2) the degree of oxygen unsaturation of the arterial blood coming from aerated lung areas;
3) the proportion of blood passing from right to left heart through unaerated channels; and
4) the oxygen consumption in the capillaries.

It is evident from the present study that the passage of blood through unaerated lung channels is of little importance in the production of anoxia or cyanosis in most types of chronic pulmonary disease.

SUMMARY

The magnitude of the shunt of blood through nonventilated lung was measured by the method of Berggren. Studies were performed in normal human beings and in patients with various types of pulmonary disease.

The mean magnitude of the shunt in 11 normal persons was 1.0 per cent of the total pulmonary blood flow.

In chronic pulmonary disease, the magnitude of the shunt of blood through the nonventilated lung was small.

Twenty patients with unilateral pulmonary disease associated with diminution in oxygen uptake of the involved lung as measured by bronchospirometry were studied. The magnitude of the shunt was small and there was no correlation between the degree of reduction of oxygen uptake and the magnitude of the shunt. This is evidence that the diminution in oxygen uptake is accompanied by a diminution in blood flow to the involved lung.

SUMARIO

Las Determinaciones del Paso de la Sangre por las Porciones No Ventiladas del Pulmón Normal y del Patológico

Con el método de Berggren se midió la magnitud de la desviación de la sangre a través del pulmón no ventilado, ejecutándose los estudios en seres humanos normales y en sujetos con varias formas de afección pulmonar.

La magnitud media de la desviación en 11 individuos normales representó 1.0 por ciento de la circulación sanguínea total por el pulmón.

En las neumopatías crónicas, la magnitud de la desviación de la sangre a través del pulmón no ventilado fué de poca cuantía.

Se estudió a 20 enfermos con afección pulmonar unilateral en que había disminución de la absorción de oxígeno en el pulmón afectado, determinada con la broncoespirometría. La desviación fué de poca cuantía y no hubo correlación entre la disminución de la toma de oxígeno y la magnitud de la desviación. Esto constituye prueba de que la disminución de la toma de oxígeno va acompañada de disminución de la circulación sanguínea al pulmón afectado.

RESUME

La mesure du débit sanguin dans les segments du poumon normal ou du poumon altéré

L'importance de la dérivation ("shunt") du sang traversant un poumon non-ventilé a été mesurée par la méthode de Berggren. Des recherches ont été faites chez des sujets normaux et chez des malades présentant diverses formes d'affection pulmonaire.

La valeur moyenne de la dérivation ("shunt") chez 11 individus normaux représentait 1% du débit sanguin pulmonaire total.

Dans les affections pulmonaires chroniques, la valeur du "shunt" du débit sanguin dans le poumon non-ventilé était réduite.

Vingt malades, atteints d'une affection pulmonaire s'accompagnant d'une réduction de l'apport d'oxygène dans le poumon intéressé, ont été étudiés par la méthode bronchospirométrique. L'importance du "shunt" était réduite et il n'existait pas de corrélation entre le degré de réduction de l'apport d'oxygène et l'importance du "shunt". Ceci constitue la preuve que la diminution de l'apport d'oxygène s'accompagne d'une diminution du débit sanguin dans le poumon altéré.

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SYNERGISTIC EFFECTS OF BCG VACCINE AND HYALURONIDASE IN GUINEA PIGS[1]

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INTRODUCTION

It is generally agreed that the tuberculin conversion rate following BCG vaccination in man is directly related to the size of the local skin reaction (1, 2). In order to approximate complete tuberculin conversion in two to three months' time after vaccination, the optimum intracutaneous dosage required is 0.05 to 0.1 mg. of BCG. Considerable fluctuations are observed in various parts of the world, even with the same batch of vaccine and more or less similar vaccination and tuberculin-testing techniques. Delay in producing tuberculin conversion necessitates prolonged isolation and often revaccination. In order to overcome this handicap, Bergqvist (3) added hyaluronidase to BCG vaccine and observed regularly accelerated and larger skin lesions and greater tuberculin conversions than with BCG vaccine alone.

Experimental and clinical use of the mucolytic enzyme has shown that small amounts facilitate the absorption of fluids by hydrolyzing hyaluronic acid, which acts as a barrier to the diffusion of invasive substances (4). Thus, Thomas and Duran-Reynals (5) observed that the "spreading factor" in testicular extracts, shown by Chain and Duthie to be hyaluronidase (6), caused extension of local tuberculosis in primarily and in secondarily infected animals, but that the ensuing generalized tuberculous lesions were most extensive in the former. These workers attributed this difference to a more rapidly developed immunity in the secondarily infected animals.

Following up this lead, Bergqvist and Packalen (7) inoculated guinea pigs and rabbits intradermally and subcutaneously with mixtures of tubercle bacilli (H37Rv) and a hyaluronidase-producing strain of *Staphylococcus*. They observed that tuberculin sensitivity developed more rapidly and strongly and the local and general tuberculous lesions more rapidly and severely in direct proportion to the enzyme production of the strain employed. In a subsequent study (8) on the effect of a purified preparation of hyaluronidase on BCG vaccine in guinea pigs, Bergqvist demonstrated that the tuberculin sensitivity developed more rapidly and strongly in these animals than in control animals. He also noted a greater frequency of excessively infiltrated and ulcerated local lesions in the hyaluronidase-treated animals than in the control animals.

The investigation was extended to a group of 95 schoolchildren approximately eight years of age who were injected intradermally with a mixture of 0.05 mg. of BCG vaccine and 0.5 viscosity units of purified hyaluronidase. Seventy-one children received only BCG vaccine. One month later, 61 per cent of the former and 35 per cent of the latter presented positive tuberculin reactions in seventy-two hours with 1.0 mg. of Old Tuberculin (OT)—a significant difference—and, when the test was repeated fifty days after vaccination, 99 per cent of the BCG-hyaluronidase group and 91 per cent of the BCG

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group had become positive tuberculin reactors. The investigator reports that a higher frequency of excessively infiltrated and ulcerated local reactions occurred in the former than in the latter groups, namely, 31 per cent of the infiltrations measuring 15 or more mm. in diameter and 30 per cent of the ulcerations in the BCG-hyaluronidase group, compared with 10 and 9.5 per cent, respectively, in the BCG group. This study was terminated fifty days after vaccination without further mention of the healing of the skin lesions.

The present study deals with the effect of purified hyaluronidase[2] on the antigenic potency of BCG vaccine in the guinea pig, with special reference to local and general lesions, degree of tuberculin sensitivity, and resistance against a challenge infection.

OBSERVATIONS

Dispersion of BCG in Hyaluronidase-Treated Guinea Pigs

Twenty normal and tuberculin-negative guinea pigs, each weighing approximately 400 gm., were injected intracutaneously in the shaved left lateral abdominal region with 0.1 ml. of phosphate buffer solution pH 7.2 which contained 1.0 mg. of freshly prepared BCG vaccine (43,600,000 viable elements) plus 50 viscosity units of hyaluronidase (Alidase). The ensuing local swelling disappeared completely within one minute, leaving only a trace of the needle puncture. A similar group of 20 normal guinea pigs received only 1.0 mg. of BCG vaccine. The local wheal was plainly visible for approximately three minutes. In order to determine the rate of dispersion of BCG from the skin to the visceral organs, 2 animals from each group were killed at intervals of one, two, four, eight, twelve, and twenty-four hours and two, five, twelve, and twenty-four days after inoculation. Materials for culture were removed with aseptic technique, making use of separate instruments for the removal of individual organs. The entire lymph nodes and spleen and approximately 1 gm. of liver and lungs were finely partitioned, homogenized, suspended in 1 ml. of phosphate buffer solution pH 7.2 containing 200 units of penicillin, and seeded out in 0.2 ml. amounts on 5 large tubes of Löwenstein-Jensen egg medium. The tubes were left in a horizontal position at 37.5°C. until excess liquid had completely evaporated, when they were sealed with rubber stoppers and left in standing position. Final reading of colonies was made in twelve weeks.

In tissues removed from the hyaluronidase-BCG group, the left inguinal, axillary, and para-iliac lymph nodes yielded cultures positive for *M. tuberculosis* one hour after inoculation; the spleen, liver, and tracheobronchial lymph nodes, twelve hours after inoculation; and the lungs, three days after inoculation. The kidneys yielded no positive cultures during the twenty-four–day period of observation.

In tissues removed from the BCG group, the left inguinal and axillary lymph nodes yielded cultures positive for *M. tuberculosis* eight hours after inoculation; the left para-iliac lymph nodes, three days after inoculation; the spleen and liver, five days after inoculation; and the tracheobronchial lymph nodes and the lungs, twelve days after inoculation. Cultures of the kidneys remained negative throughout the twenty-four–day period of observation. The hyaluronidase-BCG group tissues also yielded a greater number of colonies than the BCG group. These data are summarized in table 1.

[2] A purified brand of hyaluronidase called "Alidase" was supplied by G. D. Searle and Company.

Dispersion of BCG and Development of Tuberculin Allergy in Hyaluronidase-Treated Guinea Pigs

Two similar groups of normal guinea pigs received the same intracutaneous injections of hyaluronidase-BCG and BCG alone. In order to determine the rapidity of tuberculin sensitivity developing between the interval of BCG vaccination and diffusion of organisms to visceral organs, the site of inoculation was excised (approximately 3 by 3 cm. area) together with the underlying subcutaneous tissue and fat in 2 animals from each group at intervals of one, two, four, eight, twelve, and twenty-four hours, and two, four, six, and eight days after inoculation. The wound was closed with clips and plastic dressing. The animals were Mantoux-tested with 10 mg. of OT at weekly intervals for four weeks and with 1.0 mg. of OT the following month.

TABLE 1

DISPERSION OF BCG IN HYALURONIDASE-TREATED GUINEA PIGS
(1 Mg. of BCG and 50 Viscosity Units of Hyaluronidase Intracutaneously)

REGIONAL LYMPH NODES AND VISCERA	BCG	BCG AND HYALURONIDASE
	Positive (Colonies) Cultures	
Left inguinal nodes	8 hours	1 hour
Left axillary nodes	8 hours	1 hour
Left para-iliac nodes	3 days	1 hour
Spleen	5 days	12 hours
Liver	5 days	12 hours
Tracheobronchial nodes	12 days	12 hours
Lungs	12 days	3 days
Kidneys (sterile)	24 days	24 days

TABLE 2

TIME INTERVAL BETWEEN EXCISION OF VACCINATED SKIN AND DEVELOPMENT OF TUBERCULIN SENSITIVITY
(Mantoux Test 10 Mg. OT)

EXCISION OF SKIN	BCG	BCG AND HYALURONIDASE
	Positive Tuberculin Reactions Induration $\geq$ 6 x 6 x 1.5 mm.	
hours		
1	6 months	1 month
4	3 months	3 weeks
24	1 month	2 weeks

In the hyaluronidase-BCG group, positive tuberculin allergy was demonstrated in one month when the skin had been removed one hour after inoculation; in three weeks when excision took place four hours after inoculation; and as early as two weeks when the skin was left intact for twenty-four hours after inoculation.

In the BCG group, allergy failed to develop up to six months when the skin was removed one hour after inoculation; in three months when the skin was excised four hours after inoculation; and not before one month when the skin was left intact for twenty-four hours. The tuberculin reactions were regularly more indurated and extensive in the hyaluronidase-BCG group than in the BCG group. These data are summarized in table 2.

It appears, therefore, that a direct relationship exists between dispersion of BCG from the inoculation site and development of tuberculin allergy and that hyaluronidase accelerates the dispersion and, possibly in consequence, the development of allergy.

Intracutaneous and Transcutaneous Skin Reactions

Twelve white tuberculin-negative guinea pigs, each weighing approximately 350 gm., were injected intracutaneously in the four quadrants of the shaved left abdominal region, approximately 5 cm. apart, with 0.1 ml. of phosphate buffer solution pH 7.2, containing 50 viscosity units of hyaluronidase and 0.1, 0.01, 0.001, and 0.0001 mg. of BCG which yielded 45,200,000 viable elements per mg. On the opposite side of the abdomen, 40 punctures, approximately 3 mm. in depth, were made with the automatic apparatus through the skin moistened with three to five drops of 20 mg. of BCG per ml. plus 500 viscosity units of hyaluronidase per ml. Another similar group of animals received the same treatment except for hyaluronidase.

Immediate effects: The spreading action of hyaluronidase appeared more marked following the intracutaneous than the multiple puncture technique. The needle had hardly been withdrawn after inoculation before complete absorption of the 0.1 ml. of inoculum had taken place, leaving no measurable local swelling, while the multiple punctures presented no traumatic edema. In the absence of hyaluronidase, the 0.1 ml. of inoculum produced regularly an 8 to 10 mm. wide whitish bleb which remained visible for approximately three minutes, while the multiple punctures presented an elevated edematous circle around each perforation, increasing in size for approximately ten minutes when the traumatic effect gradually subsided until the skin appeared normal within an hour or two.

Late effects: Under the influence of hyaluronidase, within one week after inoculation the 0.1, 0.01, and 0.001 mg. of BCG-inoculated sites presented highly inflamed and indurated nodules with soft, yellow centers, while the 0.0001 mg. BCG site showed an inflamed hard nodule without central softening. The multiple puncture area presented markedly inflamed and indurated papules with occasional yellow centers. Within three weeks after inoculation, these lesions were intensified and more purulent in appearance, while the regional axillary and inguinal lymph nodes were palpably enlarged but of firm consistency. These lesions presented a maximum of fibrosis and suppuration approximately six weeks after inoculation while the regional lymph nodes continued to enlarge without softening.

Desquamation and healing began approximately eight weeks after inoculation and were nearly completed in fifteen weeks. During this interim the regional lymph nodes remained stationary in size and firm in consistency. The healed sites presented pigmentation varying from rusty-brown to yellow-purple. As late

as eighteen months after inoculation, 2 intracutaneously and transcutaneously inoculated guinea pigs still presented desquamating and pigmented skin lesions and enlarged regional lymph nodes of firm consistency (to be described later).

In the absence of hyaluronidase, within one week after inoculation of 0.1 and 0.01 mg. of BCG, the inoculated sites regularly showed moderately inflamed and suppurating nodules, and the 0.001 mg. BCG site presented a hardened nodule while the 0.0001 mg. BCG site showed no lesion. The multiple puncture area showed discretely inflamed and infiltrated papular elevations without suppurating centers. Three weeks after inoculation these lesions had reached their maximum development, and desquamation and healing were nearly completed in five to eight weeks after inoculation. The regional axillary and inguinal lymph

TABLE 3

AVERAGE MAXIMUM DIAMETER OF SKIN LESIONS OR PAPULES AFTER INJECTION OF GRADED DOSES OF BCG ALONE OR MIXED WITH 50 VISCOSITY UNITS OF HYALURONIDASE (12 Animals in Each Group)

DOSE OF BCG	AREA OF SKIN LESION (MM.)							
	1 Week		3 Weeks		8 Weeks		12 Weeks	
	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase
mg.								
0.1	5.7	12.5*	6.5	14.3*	3.2	14.4*	0	5.6
0.01	4.2	7.3*	5.3	8.4*	0	9.5	0	3.9
0.001	2.7	4.7*	3.2	5.4*	0	5.1	0	1.4
0.0001	0	1.5	0	1.7	0	1.5	0	0
20 per ml. multiple puncture	1.9	3.2*	2.8	3.9*	1.3	4.1*	0	1.5

* Significantly different from untreated group at $P \leq 0.01$ level.

nodes were only slightly enlarged and of soft consistency. Pigmentation was rarely observed in the inoculated sites.

The quantitative data on the skin lesions observed one, three, eight, and twelve weeks after inoculation are presented in table 3. It is apparent that the size of reactions produced by graded doses of BCG was significantly greater and of a more protracted character in the hyaluronidase-treated than in the control BCG vaccine group.

Tuberculin sensitivity reactions: Twenty-four white tuberculin-negative guinea pigs, each weighing approximately 350 gm., were inoculated subcutaneously in the left hind leg with 10 mg. of freshly prepared BCG vaccine (48,120,000 viable elements per mg.) and 500 viscosity units of hyaluronidase. An equal number of animals received only 10 mg. of BCG vaccine in a similar fashion. It was noted that the absorption of the BCG-hyaluronidase inoculum was almost instantaneous, while the BCG vaccine inoculum remained palpable up to three minutes. The rapidity and degree of tuberculin sensitivity were determined at two-, four-, eight-, and twelve-week intervals by Mantoux tests with graded doses of OT freshly diluted in phosphate buffer solution pH 7.2 and injected into the shaved abdominal area. Examina-

tions were made regularly after forty-eight hours. The requirement for a positive reaction was definite induration or edema irrespective of erythema. The two maximum diameters of indurated area were multiplied by half the thickness of skin folded between caliper prongs; for example, 5 by 5 by 1.5 mm. = 37.5 mm.[3]

The average measurements of tuberculin sensitivity are presented in table 4. It is apparent that the rapidity and degree of allergy are significantly greater in the BCG-hyaluronidase group than in the BCG group even two weeks after inoculation, when it was observed that the former group reacted with 10.0, 1.0, and 0.1 mg. of OT while the latter group reacted only with 10.0 mg. of OT. The same phenomenon was observed in tests performed four, eight, and twelve weeks after inoculation with gradually higher dilutions of OT. The BCG-hyaluronidase

TABLE 4

AVERAGE MAXIMUM AREA OF INDURATION WITH GRADED DOSES OF OLD TUBERCULIN IN GUINEA PIGS INOCULATED SUBCUTANEOUSLY WITH 10 MG. OF BCG ALONE OR MIXED WITH 500 VISCOSITY UNITS OF HYALURONIDASE

(24 Animals in Each Group)

DOSE OF TUBERCULIN	AREA OF SKIN INDURATION (MM.²)							
	2 Weeks		4 Weeks		8 Weeks		12 Weeks	
	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase
mg.								
10.0	24	580*	206	615*	246	843*	337	968*
1.0	0	118	64	345*	132	211*	320	543*
0.1	0	78	36	115*	49	168*	344	489*
0.01	0	0	0	32	0	37	121	256*
0.001	0	0	0	0	0	0	0	65

* Significantly different from untreated group at $P \leq 0.01$ level.

group reacted well with 0.001 mg. of OT, while no reactions were elicited with that dose in the BCG group. It appears definite, therefore, that hyaluronidase both hastens and intensifies tuberculin sensitization in BCG-vaccinated guinea pigs.

Tuberculogenic Potency and Virulence of the BCG-Hyaluronidase Mixture

In a three-year control study (9, 10) of weekly lots of BCG vaccine, it was proved that potency and virulence were relatively stable. This was established by determining the hyperplastic changes induced in regional and distal lymph nodes and in the spleen, liver, and lungs of guinea pigs injected subcutaneously in the left hind leg with 10 mg. of BCG and killed three and six months afterwards. In view of the accelerated and intensified skin lesions and tuberculin sensitivity produced by the BCG-hyaluronidase mixture above, it was decided to investigate whether visceral changes induced by 10 mg. of BCG would be enhanced by the BCG-hyaluronidase mixture.

For this purpose 72 normal and tuberculin-negative guinea pigs, weighing 342 plus-minus 30.94 gm., were divided into two equal groups, the animals in one of which were injected subcutaneously in the left hind leg with 10 mg. of freshly prepared BCG vaccine (43,300,000 living elements per mg.), and those in the other group with the same dose of BCG plus 500 viscosity units of hyaluronidase. It was noted that the BCG-hyaluronidase inoculum was instantly absorbed following injection and that the BCG inoculum remained palpable for approximately three minutes.

During the following six-month observation period, no difference in the growth curve of the two groups of animals or in their general condition was observed. Development of the primary abscess seemed markedly accelerated in the BCG-hyaluronidase group up to three weeks after inoculation, when resorption gradually set in and rendered the site firm in consistency. In the BCG group the abscess grew gradually, with a tendency to softening and fluctuation from one month after inoculation. Thereafter, the lesion remained almost static until the animal was killed six months after inoculation. One month after inoculation, the regional lymph nodes were of greater dimension and firmer consistency in the BCG-hyaluronidase than in the BCG group and, with the passing of time, the softening of these nodes was more marked in the former than in the latter group.

Three spontaneous deaths occurred in the BCG-hyaluronidase group (69, 125, and 183 days after inoculation) and none in the BCG group. The 3 which died were markedly underweight and presented more or less similar macroscopic and microscopic generalized tuberculous lesions as follows:

Heavily fibrosed and partly calcified caseonecrotic abscesses in the left thigh, markedly enlarged and centrally caseonecrotic regional and distal lymph nodes, greatly enlarged and heavily tuberculized spleen, miliary gray tubercles and marginal infarcts in the liver, and croupous pneumonitis with miliary gray tubercles in one or more lobes. The granulomas showed various degrees of caseonecrosis surrounded by a mantle of epithelioid cells, occasionally giant cells of the Langhans' type, and numerous acid-fast rods.

Cultures of these lymph nodes and the visceral organs gave rise to numerous rough colonies of acid-fast bacilli. Reinoculations up to 10 mg. of the cultures into normal guinea pigs failed to produce generalized tuberculosis during the following six months, and the gross pathologic picture was that of the routinely BCG-inoculated animals necropsied three and six months after similar inoculation. Thus, there was no evidence of alteration of virulence in the organism.

Comparative Weights of Spleen and Lymph Nodes in BCG-Hyaluronidase and BCG Groups

Twelve guinea pigs in the BCG-hyaluronidase and 12 in the BCG groups were killed at intervals of one, three, and six months after inoculation in order to observe the lymph nodes and viscera and to assess quantitatively the differences between the two groups. Necropsy and weighing procedures were the same as previously reported (9, 10). In table 5 is summarized the average weight of individual regional and distal lymph nodes, as well as of the total pooled lymph nodes, and of the spleen, liver, and lungs.

One month after inoculation the regional superficial inguinal and the para-iliac lymph nodes in the BCG-hyaluronidase group were significantly larger than the corresponding nodes in the BCG group. Three months after inoculation similar hyperplastic changes had taken place in the regional deep inguinal, the distal tracheobronchial nodes, as well as in the total pooled lymph nodes, and in the spleen. The increments in weight persisted in the animals killed six months after inoculation.

It appears, therefore, that the tuberculogenic potency of BCG is activated by hyaluronidase to produce significantly greater cellular reaction than is normally observed with BCG vaccine alone and that occasionally the generalized reaction may prove fatal to the animal.

TABLE 5

AVERAGE WEIGHT OF LYMPH NODES, SPLEEN, LIVER, AND LUNGS OF GUINEA PIGS INOCULATED SUBCUTANEOUSLY IN LEFT HIND LEG WITH 10 MG. OF BCG ALONE OR MIXED WITH 500 VISCOSITY UNITS OF HYALURONIDASE AND KILLED AFTER ONE, THREE, AND SIX MONTHS

(12 Animals in Each Group)

MATERIAL	AVERAGE WEIGHT (GM.)					
	1 Month		3 Months		6 Months	
	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase	BCG	BCG and Hyaluronidase
	gm.					
Animals' body weight	416	404	646	629	806	814
Left superficial inguinal nodes	0.17	0.29*	0.19	0.34*	0.23	0.39*
Left deep inguinal nodes	0.04	0.06	0.05	0.08*	0.05	0.08*
Left para-iliac nodes	0.12	0.22*	0.16	0.27*	0.18	0.25*
Tracheobronchial nodes	0.27	0.34	0.29	0.38*	0.31	0.44*
Total lymph nodes	1.03	1.39	1.09	1.63*	1.14	1.94*
Spleen	0.68	0.76	0.76	0.88*	0.79	0.93*
Liver	34.18	35.09	36.10	37.06	38.60	40.43
Lungs	4.18	4.39	4.59	5.12	5.06	5.93

* Significantly different from untreated group at $P \leq 0.01$ level.

Persistence of Cutaneous Lesions

Mention should be made of the post-mortem findings in 2 guinea pigs kept under observation for eighteen months after the intracutaneous and transcutaneous inoculations with graded doses of BCG detailed earlier in this paper. The persistence of the multiple puncture lesions and excessive scar tissue in the sites inoculated with 0.1 and 0.01 mg. of BCG plus 50 viscosity units of hyaluronidase, as well as excessively hypertrophied regional lymph nodes, prompted the writer to delay sacrificing these animals. Their body weight had remained almost stationary, approximately 500 gm., throughout.

Dissection disclosed heavily fibrosed and partly calcified regional and distal lymph nodes with caseonecrotic centers. The spleen was larger than normal and consisted principally of infarcted tissue; the liver showed numerous coalescing

gray-yellow tubercles and marginal infarcts, and the lungs were studded with gray tubercles. Microscopic sections revealed that the skin lesions contained small epithelioid tubercles extending into the subcutaneous tissue and the muscle; the regional inguinal and para-iliac lymph nodes showed massive caseonecrotic and calcareous disease, and the distal lymph nodes presented a lesser degree of caseonecrosis with tendency to fibrosis and calcification; the spleen showed extensive necrotic tuberculosis; the liver showed a moderate number of epithelioid tubercles which were rarely necrotic; and the lungs showed occasional tuberculous foci consisting essentially of epithelioid cells, macrophages, and lymphocytes with rare giant cells. Cultures of the lymph nodes and the visceral organs yielded an abundance of rapidly developing rough colonies of acid-fast bacilli which, on reinoculation into normal guinea pigs, gave rise to localized but not generalized tubercles of the BCG-produced variety. In the writer's experience with BCG infection in guinea pigs, such protracted persistence of cutaneous lesions with generalized tuberculosis has not previously been observed.

Immunogenic Response to the BCG-Hyaluronidase Inoculum

Bergqvist (3) postulated that, inasmuch as the BCG-hyaluronidase inoculum accelerated and intensified both the vaccination lesions and the tuberculin sensitization, it might enhance and prolong specific resistance against tuberculosis in man. No experimental proof was presented, however, to support this supposition. In order to assess the immune response to the BCG-hyaluronidase inoculum under controlled conditions, the following experiment was performed:

Twelve normal and tuberculin-negative guinea pigs, each weighing 351 plus-minus 40.29 gm., were immunized with 10 mg. of BCG vaccine (52,100,000 viable elements per mg.) inoculated subcutaneously into the left thigh. Another similar group of animals, each weighing 345 plus-minus 28.16 gm., were given by the same route 500 viscosity units of hyaluronidase plus the same dose of BCG vaccine. A third similar group of animals, each weighing 328 plus-minus 37.10 gm., were kept uninoculated for control purposes. Three months after inoculation, these 36 animals were given a challenge inoculation of 0.005 mg. of H37Rv in the right thigh. This dose yielded 3,105 living elements (colonies) in a series of subcultures.

Death from generalized and advanced tuberculosis occurred in 9 control animals (75 per cent) between 72 and 107 days after inoculation; in 2 animals in the BCG group, 91 and 105 days after the challenge inoculation; and in 3 animals in the BCG-hyaluronidase group, 89, 96, and 104 days after inoculation. The surviving animals were killed between 108 and 112 days after the challenge inoculation.

The post-mortem findings are summarized in table 6. It may be seen that the animals' body weight was approximately the same in the BCG-hyaluronidase and BCG groups at the time of necropsy and that it differed significantly from that of the control animals. Macroscopically, no significant differences were observed between the two immunized groups. The quantitative assessment of lymphonodular tuberculous hyperplasia, however, indicated that the right re-

gional nodes in the BCG-hyaluronidase group were significantly lighter in weight than in the BCG group (their left counterparts were directly affected by the BCG-hyaluronidase inoculum alone as shown earlier and, as such, were useless as criterion for determination of specific resistance). Nevertheless, the total weight of 17 sets of nodes from each animal proved to be significantly less in the BCG-hyaluronidase than in the BCG group of animals. The visceral organs showed no significant differences in weight in the 2 immunized groups although both differed from the control animals.

TABLE 6

POST-CHALLENGE FINDINGS IN GUINEA PIGS IMMUNIZED WITH 10 MG. OF BCG ALONE OR MIXED WITH 500 VISCOSITY UNITS OF HYALURONIDASE

(Twelve Animals in Each Group)

MATERIAL	BCG	BCG AND HYALURONIDASE	CONTROLS
Animals' weight at necropsy, gm.	795*	805*	676
No gross tuberculous lesions	7	8	0
Localized tuberculous lesions	3	3	3
Generalized tuberculous lesions	2	1	9
Right superficial inguinal nodes, gm.	0.29*	0.20*	0.85
Right deep inguinal nodes, gm.	0.10*	0.09*	0.36
Right para-iliac nodes, gm.	0.61*†	0.27*	1.36
Periportal node, gm.	0.19*	0.11*	0.87
Left tracheobronchial nodes, gm.	0.36*	0.33*	0.94
Right tracheobronchial nodes, gm.	0.37*†	0.28*	0.99
Right knee node, gm.	0.13*†	0.03*	1.37
Retrorenal nodes, gm.	0.23*†	0.14*	0.35
Total lymph nodes, gm.	3.53*†	2.33*	9.61
Spleen, gm.	1.09*	1.15*	3.71
Liver, gm.	39.90*	36.78*	44.19
Lungs, gm	7.17*	6.79*	8.43

* Significantly different from controls at $P \leq 0.01$ level.

† Significantly different from hyaluronidase-BCG group at $P \leq 0.01$ level.

SUMMARY

Hyaluronidase accelerates and intensifies the degree of local and general dispersion of BCG vaccine, the production of vaccination skin lesions and tuberculin sensitivity, and occasionally induces generalized tuberculous infection without an accompanying enhancement of the virulence of the vaccine.

Hyaluronidase stimulates BCG vaccine to produce a slightly higher degree of specific resistance to tuberculous infection than is produced by BCG vaccine alone.

The synergistic action of hyaluronidase and BCG vaccine produces protracted ulcerative skin lesions, lymphoglandular hyperplasia, and occasional generalized tuberculous infection in guinea pigs; hence, the combination should not be used in man.

SUMARIO

Efectos Sinérgicos de la Vacuna BCG y la Hialuronidasa en los Cobayos

La hialuronidasa acelera e intensifica la dispersión local y general de la vacuna BCG, la producción de lesiones cutáneas de la vacunación y de sensibilidad a la tuberculina y provoca en ocasiones infección tuberculosa generalizada sin aumentar la virulencia de la vacuna.

La hialuronidasa estimula la vacuna BCG a producir una resistencia específica ligeramente mayor a la infección tuberculosa que la vacuna BCG sola.

La acción sinérgica de la hialuronidasa y la vacuna BCG produce prolongadas lesiones cutáneas ulceradas, hiperplasia linfoganglionar y a veces infección tuberculosa generalizada en los cobayos, y de ahí que no deba usarse la combinación en el hombre.

RESUME

Action synergique du vaccin BCG et de l'hyaluronidase chez le cobaye

L'hyaluronidase accélère et intensifie le degré de dispersion locale et générale du vaccin BCG, la production des lésions cutanées de la vaccination et la sensibilité à la tuberculine; occasionnellement, elle provoque l'infection tuberculeuse généralisée sans amplifier la virulence du vaccin.

L'hyaluronidase exerce une action stimulante sur le vaccin BCG, qui se traduit par le degré légèrement plus élevé de la résistance spécifique à l'infection tuberculeuse que de celle obtenue avec le BCG seul.

Chez le cobaye, l'action synergique de l'hyaluronidase et du vaccin BCG produit des lésions cutanées ulcéreuses prolongées, l'hyperplasie des ganglions lymphatiques et parfois l'infection tuberculeuse généralisée; en conséquence, cette association ne doit pas être faite chez l'homme.

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SOME PHARMACOLOGICAL OBSERVATIONS ON ISONIAZID AND IPRONIAZID[1, 2]

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INTRODUCTION

Since a number of drugs such as morphine, barbiturates, and closely related drugs will be administered along with isoniazid clinically, it seemed important to determine the effect of isoniazid on certain actions of some of these drugs. Moreover, as it has been reported that tremor is the most frequently encountered side-effect in patients receiving isoniazid (1, 2), various drugs were studied for their preventive action in rabbits which had been given a convulsive dose of isoniazid. Some of these drugs have been reported to be effective in preventing nicotine-induced convulsion (3, 4, 5, 6).

In view of the evidence as revealed in a clinical report that isoniazid and its isopropyl derivative might have some effect on the action of digitalis, the actions of the former drugs were studied in the isolated mammalian hearts, and also the possible effect of isoniazid on the lethal action of digitoxin was investigated. The present paper is a report of the findings of these various experimental studies.

EXPERIMENTAL

For all the experiments on rats, groups of 10 rats, either male or female, weight range 125 to 175 gm., and suitably randomized, were used on the respective doses in each experiment. The solutions of the different drugs were made up in distilled water and the volume injected did not exceed 1 ml. The rats were placed in individual cages immediately after injection of the barbiturate and the interval between time of dosing and return of the righting reflex (for convenience called sleeping time) was noted (7). The other pertinent data with respect to these experiments are shown in the table.

For the anti-tremor experiments, 10 rabbits ranging in weight from 0.5 to 2.0 kg. were used on each of the two control groups as well as on each of the treated groups. Isoniazid was given intravenously (100 mg. per kg.), and the anti-tremor drugs were given subcutaneously immediately after the injection of isoniazid. For the isolated heart experiments the apparatus and procedure previously reported (8) were used.

The three barbituric acid derivatives as sodium salts, morphine as sulfate, *d*-tubocurarine as chloride, and atropine as sulfate were used in these experiments and the weights of these drugs used refer to the respective salts.

RESULTS

Effect on sleeping time: In the experimental results reported in table 1, the effects of isoniazid on the sleeping times induced by hexobarbital, pentobarbital, and secobarbital are shown. In some cases it is quite evident that the pretreat-

[1] From the Pharmacology Section, Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada.

[2] Presented in part at the Annual Meeting of the Canadian Physiological Society, October 10, 1952, at Quebec City.

ment of rats with isoniazid has prolonged the sleeping time; in others, the effect is not so noticeable. However, an analysis of variance on the data from table 1, as shown in table 2, column 4, demonstrates that the pretreatment of rats with isoniazid has significantly prolonged the sleeping time in every case.

Effect on mortality: In table 3 the results of two experiments in which rats were pretreated with isoniazid are shown. The various doses of isoniazid had no appreciable effect on the mortality rates induced by morphine or meperidine as shown by a chi-square test.

TABLE 1

EFFECT OF ISONIAZID ON THE SLEEPING TIMES INDUCED BY HEXOBARBITAL, PENTOBARBITAL, AND SECOBARBITAL IN MALE AND FEMALE RATS

ISONIAZID, MG/KG, INTRAPERITONEALLY		0	2	4	8	16	32	64
		Mean Sleeping Times in Minutes						
Hexobarbital, mg/kg, intraperitoneally,	70.0	27	31	27	30	37	38	
male rats; injected sixty minutes after	87.5	30	33	43	34	40	54	
isoniazid	109.4	43	37	51	50	55	69	
Secobarbital, mg/kg, intraperitoneally,	60.0	60	60	73	85	125	181	
female rats; injected 60 minutes after	75.0	94	90	112	111	170	206	
isoniazid	94.0	114	122	132	165	192	244	
Pentobarbital, mg/kg, intraperitoneally,	25.0	56				80	100	134
male rats; injected 45 minutes after	30.0	72				96	138	170
isoniazid	36.0	77				101	145	198
Pentobarbital, mg/kg, intraperitoneally,	20.0	112			132	152	160	202
female rats; injected 45 minutes after	24.0	130			163	189	201	217
isoniazid	28.8	171			199	217	277	265
Secobarbital, mg/kg, intraperitoneally,	26.0	48			43	56	80	
male rats; injected 60 minutes after	32.5	50			68	96	89	
isoniazid	40.6	70			82	110	124	
Secobarbital, mg/kg, intraperitoneally,	19.2	84			101	138	153	
female rats; injected 60 minutes after	24.0	119			176	168	244	
isoniazid	30.0	177			215	200	278	

Anticonvulsant drugs: In table 4 are shown the results of a number of antitremor drugs on convulsions elicited by isoniazid. As shown in the table, 50 per cent of each of the two groups of control animals convulsed. In these experiments, pentobarbital was found to be most effective in blocking the convulsion. A hypnotic effect was observed in all rabbits which received the larger dose of pentobarbital but in none of those which received the smaller dose. A highly significant effect was observed with 92 GT[3] at the higher dose level and a non-

[3] Beta-diethylaminoethyl (1-hydroxycyclopentyl) phenyl acetate HCl.

significant effect at the lower dosage level. Tubocurarine, Diparcol, and Panparnit showed significant effects at both dosage levels. The effects of Dibenamine

TABLE 2

ANALYSIS OF VARIANCE ON DATA FROM TABLE 1

VARIANCE DUE TO	ISONIAZID		LINEAR REGRESSION ON ISONIAZID		DEPARTURE FROM LINEARITY: ISONIAZID		BARBITURATE		LINEAR REGRESSION ON LOG-DOSE: BARBITURATE		DEPARTURE FROM LINEARITY: BARBITURATE		INTERACTION: ISONIAZID VS. BARBITURATE		WITHIN GROUPS (ERROR)	
	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square	d.f.	Mean Square
Isoniazid and hexobarbital; male rats	5	0.1750 ++	1	0.8127 ++	4	0.0156	2	0.6277 ++	1	1.2546 ++	1	0.0007	10	0.0144	152	0.0260
Isoniazid and hexobarbital; female rats	5	0.7258 ++	1	3.4527 ++	4	0.0441 ++	2	0.8618 ++	1	1.7089 ++	1	0.0147	10	0.0170	153	0.0072
Isoniazid and pentobarbital; male rats	3	0.8176 ++	1	2.3454 ++	2	0.0537	2	0.3073 ++	1	0.5478 ++	1	0.0667	6	0.0048	85	0.0171
Isoniazid and pentobarbital; female rats	4	0.2566 ++	1	0.8390 ++	3	0.0624 +	2	0.4297 ++	1	0.8482 ++	1	0.0112	8	0.0105	135	0.0158
Isoniazid and secobarbital; male rats	3	0.3586 ++	1	0.9973 ++	2	0.0393	2	0.5968 ++	1	1.1932 ++	1	0.0004	6	0.0293	105	0.0129
Isoniazid and secobarbital; female rats	3	0.3235 ++	1	0.9218 ++	2	0.0245	2	0.7925 ++	1	1.5764 ++	1	0.0085	6	0.0326	100	0.0181

+—significant at the 5 per cent point.
++—significant at the 1 per cent point.

TABLE 3

EFFECT OF ISONIAZID ON THE ACUTE TOXICITIES OF MORPHINE AND MEPERIDINE IN MALE AND FEMALE RATS

ISONIAZID, MG/KG, INTRAPERITONEALLY		0	12	24	48	96
		Per Cent Mortality				
Morphine, mg/kg, intraperitoneally, female rats; given one hour after isoniazid.	200	50	20	30	60	60
	230	60	40	60	70	70
	265	80	60	70	90	90
Meperidine, mg/kg, intraperitoneally, male rats; given one-half hour after isoniazid.	73	30		30	50	40
	82	40		50	60	60
	92	50		60	70	70

and Artane were significant in the higher dose groups. Atropine, 93 GT[4], and Thephorin did not exert any significant effect.

In table 5 are shown the results of two experiments in which several groups of rats were pretreated with various doses of isoniazid and then injected with differ-

[4] Beta-diethylaminoethyl (1-hydroxycyclohexyl) phenyl acetate HCl.

ent doses of hexobarbital. The animals received 374 to 470 mg. per kg. in the first experiment and 640 to 1,000 mg. per kg. in the second experiment. It can be observed that the mortality rates were significantly reduced by the barbitu-

TABLE 4

EFFECT OF VARIOUS DRUGS ON THE CONVULSION PRODUCED BY ISONIAZID, 100 MG. PER KG. INTRAVENOUSLY

DRUG	DOSE	EFFECT: NUMBER CONVULSED / NUMBER INJECTED	χ^2	DOSE	EFFECT: NUMBER CONVULSED / NUMBER INJECTED	χ^2
Control		5/10			5/10	
	mg/kg			*mg/kg*		
1. Pentobarbital	5.0	0/10	10.0*	20.0	0/10	10.0*
2. 92 GT†	5.0	2/10	3.6	20.0	0/10	10.0*
3. Tubocurarine	0.1	1/10	6.2‡	0.2	1/10	6.2‡
4. Diparcol	5.0	1/10	6.2‡	20.0	1/10	6.2‡
5. Panparnit	5.0	1/10	6.2‡	20.0	1/10	6.2‡
6. Dibenamine	4.0	3/10	1.6	16.0	1/10	6.2‡
7. Artane	5.0	4/10	0.4	20.0	1/10	6.2‡
8. Atropine	5.0	2/10	3.6	20.0	3/10	1.6
9. 93 GT§	5.0	4/10	0.4	20.0	2/10	3.6
10. Thephorin	5.0	3/10	1.6	20.0	3/10	1.6

* Significant at $P = 0.005$.
† Beta-diethylaminoethyl (1-hydroxycyclopentyl) phenyl acetate HCl.
‡ Significant at $P = 0.05$.
§ Beta-diethylaminoethyl (1-hydroxycyclohexyl) phenyl acetate HCl.

TABLE 5

EFFECT OF HEXOBARBITAL ON THE ACUTE TOXICITY OF ISONIAZID IN FEMALE RATS

HEXOBARBITAL, MG/KG, INTRAPERITONEALLY; GIVEN 40 MINUTES AFTER ISONIAZID		0	4	8	16
		Per Cent Mortality			
Isoniazid, mg/kg, intraperitoneally; female rats	375	10	0	0	0
	420	50	0	0	0
	470	100	20	0	0
HEXOBARBITAL, MG/KG, INTRAPERITONEALLY; GIVEN 20 MINUTES AFTER ISONIAZID		0	60	75	94
		Per Cent Mortality			
Isoniazid, mg/kg, intraperitoneally; female rats	640	100	0	0	20
	800	100	10	20	30
	1,000	100	10	0	50

rates at all dosage levels. The greater mortality rates observed at the highest dose level of the barbiturate in the second experiment may be caused by an additive toxicity of the hypnotic drug.

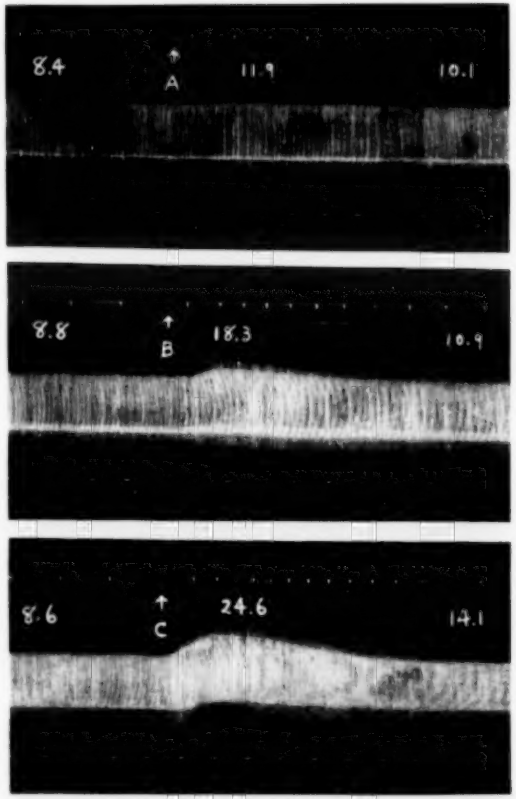

FIG. 1

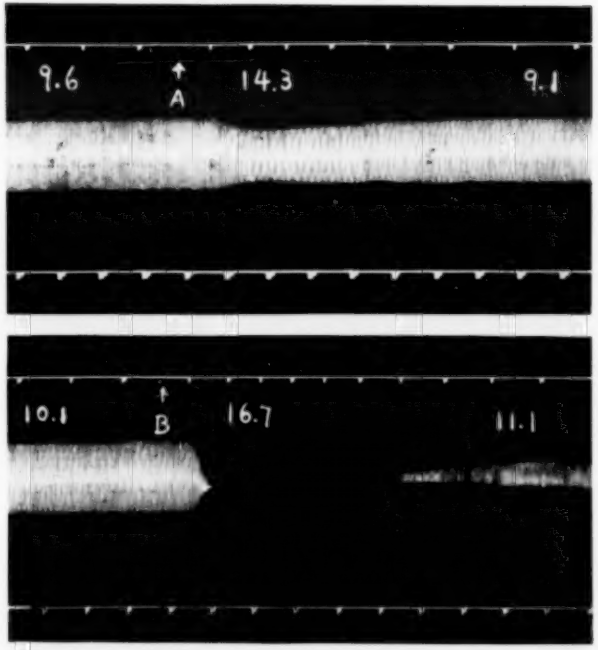

FIG. 2

FIG. 1. In each section of this figure and figure 2, the upper line is the record of coronary flow. During the period of each interval a total of 4 cc. of perfusate flowed through the coronaries. The figures under this line indicate the rate of coronary flow in cubic centimeters per minute. The middle tracing shows heart beat, systole up. The lower line indicates time. Each interval represents ten seconds. Is oniazid, 10, 50, and 250 mg., was given at *A*, *B*, and *C*, respectively.

FIG. 2. Iproniazid, 2 and 10 mg., was given at *A* and *B*, respectively.

Experiments on rabbit heart and with digitoxin: In figure 1 the effects of isoniazid in the isolated rabbit heart are shown. At *A*, an injection of 10 mg. of isoniazid was given. There was a slight increase in heart rate and a definite increase in coronary flow. At *B* and *C*, injections of 50 and 250 mg. of isoniazid were given. Both the heart stimulating and coronary dilator actions were more marked as the dose was increased. The effects of iproniazid are shown in figure 2; 2 and 10 mg. were given at *A* and *B*, respectively. Depression of heart contraction and increase in coronary flow were evident in both cases. Experiments performed in the isolated dog heart showed similar effects.

The action of digitoxin was studied in two groups of pigeons; one was pretreated with isoniazid and the other served as a control (table 6). Isoniazid, 50 mg. per kg., was given intravenously thirty minutes before the infusion of digitoxin (U.S.P. XII method used). The average lethal dose of digitoxin in the control and in the pretreated group was approximately the same.

TABLE 6

THE EFFECT OF THE PRETREATMENT OF PIGEONS WITH ISONIAZID, 50 MG. PER KG. INTRAVENOUSLY, ON THE MEAN LETHAL DOSE OF DIGITOXIN

MEAN LETHAL DOSE MG. PER KG. PLUS MINUS STANDARD DEVIATION, CONTROL	MEAN LETHAL DOSE MG. PER KG. PLUS MINUS STANDARD DEVIATION, PRETREATED
0.259 ± 0.008	0.272 ± 0.013

6 pigeons in each group.

DISCUSSION

The results of these experiments indicate that prior dosing of rats with isoniazid significantly prolongs the sleeping time induced by hexobarbital, pentobarbital, and secobarbital, and prior dosing of rats with isoniazid has no effect on the mortality rates of morphine and meperidine. Hexobarbital has a significant protective effect on the lethal action of isoniazid. Thus, the toxic actions of isoniazid in the rat as well as the rabbit are protected by barbiturates.

The motor excitatory effect of isoniazid is probably central in origin since it has been shown that it has no effect on the peripheral motor organs (9). It is of interest to note that, while isoniazid, unlike nicotine, is essentially inactive pharmacodynamically (9), it possesses a stimulating action on the central nervous system similar to that of nicotine. This is shown by the fairly close agreement between the effects of various antagonists on the convulsions induced by these two agents.

While the effectiveness of the barbiturates (central nervous system depressants) in blocking the motor excitatory action of isoniazid, observed in the rabbit as well as the rat, is to be expected, it is surprising to find that the hypnotic effect of barbiturates was prolonged by isoniazid in the rat. No attempt was made in the present study to determine the mechanism underlying this effect. However, the following factors may be responsible: (*1*) Isoniazid may inhibit the destruction of barbiturates in the body; (*2*) isoniazid at low dosage levels is

without a stimulating action and may increase the sensitivity of the central nervous system to some substances such as barbiturates; and (*3*) isoniazid may increase the cerebral blood flow and thus increase the barbiturate concentration in the brain.

Since isoniazid and iproniazid are chemically related to nicotinic acid, the coronary dilator action of which has been demonstrated (10), it is not surprising to find that these compounds also showed such an action.

SUMMARY

Prior intraperitoneal injection of rats (thirty to sixty minutes) with isoniazid was found to prolong the sleeping time induced by hexobarbital, pentobarbital, and secobarbital in male and female rats.

The mortality rates due to morphine and meperidine were not affected by prior intraperitoneal injection of isoniazid.

Hexobarbital significantly reduced the mortality rate produced by isoniazid in rats.

Pentobarbital, 92 GT, tubocurarine, Diparcol, Panparnit, Dibenamine, Artane, atropine, 93 GT, and Thephorin were studied for their anti-tremor action on isoniazid-induced convulsions in rabbits. With the exception of atropine, 93 GT, and Thephorin, the others had a significant effect.

In the isolated perfused mammalian heart, isoniazid showed a positive, and iproniazid a negative, inotropic action. Both increased the rate of coronary flow. Isoniazid given intravenously thirty minutes before the infusion of digitoxin did not significantly affect the lethal dose of the latter in the pigeon.

SUMARIO

Algunas Investigaciones Farmacológicas de la Isoniacida y la Iproniacida

Según se observó, la previa inyección intraperitoneal (treinta a sesenta minutos) de isoniacida prolongó la duración del sueño producido por el hexobarbital, el pentobarbital y el secobarbital en las ratas de ambos sexos.

La mortalidad debida a la morfina y la meperidina no fué afectada por la previa inyección intraperitoneal de isoniacida.

El hexobarbital rebajó en forma significativa la mortalidad producida por la isoniacida en las ratas.

El pentobarbital, el 92 GT, la tubocurarina, el Diparcol, el Panparnit, la Dibenamina, la Artana, la atropina, el 93 GT y la Teforina fueron estudiados con respecto a su acción antitemblor en las convulsiones provocadas con isoniacida en los conejos. Exceptuados la atropina, el 93 GT y la Teforina, todos ejercieron efecto significativo.

En el corazón de mamífero aislado y perfundido, la isoniacida reveló una acción inotropa positiva y la iproniacida, una acción negativa. Ambas acrecentaron la velocidad de la circulación coronaria. La isoniacida administrada endovenosamente treinta minutos antes de la inyección de digitoxina no afectó en forma importante la dosis letal de la última en la paloma.

RESUME

Quelques investigations pharmacologiques sur l'Isoniazide et l'Iproniazide

L'injection intra-péritonéale d'isoniazide au rat, 30 à 60 minutes avant l'anesthésie par l'hexobarbital, le pentobarbital et le sécobarbital, a prolongé la durée du sommeil chez le rat mâle ou femelle.

Les taux de mortalité dus à l'intoxication par la morphine et la mépéridine n'étaient pas modifiés par l'injection intrapéritonéale préalable d'isoniazide.

L'hexobarbital réduisait de façon significative le taux de mortalité déterminé par l'isoniazide chez le rat.

Le pentobarbital, le 92 GT, la tubocurarine, le Diparcol, le Panparnit, la Dibénamine, l'Artane, l'atropine, le 93 GT et la Théphorine ont été étudiés en ce qui concerne leur action antispasmodique sur les convulsions produites par l'isoniazide chez le lapin. Sauf l'atropine, le 93 GT et la Théphorine, l'efficacité des autres préparations a été démontrée.

Sur le coeur de mammifère isolé, perfusé, l'action inotrope a été positive avec l'isoniazide et négative avec l'iproniazide. Ces deux produits augmentaient le débit coronaire. Dans l'expérimentation chez le pigeon, l'iproniazide administrée par voie intraveineuse trente minutes avant la perfusion de digitoxine ne modifiait pas de façon significative la dose léthale de cette dernière.

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NITROGEN METABOLISM AND FOOD INTAKE IN NONTUBERCULOUS PATIENTS RECEIVING ISONIAZID[1, 2]

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INTRODUCTION

The use of isoniazid in tuberculous patients has been accompanied by marked increase in appetite and increments in body weight which have been unusual in comparison with other forms of therapy for tuberculosis (1). These reports have led to the speculation that such results may, in part, be the result of an anabolic effect of the drug independent of its effect in combating tuberculous infection. In an attempt to study this possibility, studies of nitrogen balance have been conducted in 3 patients who were receiving isoniazid. In addition, in an effort to study the question of whether there is a specific appetite-stimulating effect inherent in isoniazid, one patient has been studied during a regimen of limited food selection in order to determine whether or not there was an increased nutrient intake while receiving the drug.

METHODS

Three patients with nontuberculous disease were studied before, during, and after the administration of isoniazid.[3]

H. S., a 60-year-old male convalescing from pneumonia, received a constant diet containing 50 gm. of protein and 2,000 calories. This was given for four control days, during ten days of administration of isoniazid, 5 mg. per kg. of body weight, and then continued for four more days.

E. R., a 38-year-old female with the syndrome of chronic hepatitis but with normal liver function tests and normal hepatic tissue obtained by biopsy, was similarly studied for a six-day control period. She then received isoniazid, 4 mg. per kg. of body weight, for six days followed by a subsequent five-day control period. She was offered a diet containing 68 gm. of protein and 2,300 calories daily. There was slight variation in the daily food intake in this patient, but this probably did not significantly affect the nitrogen balance studies.

G. H., a 30-year-old male with nontropical sprue, was studied in slightly different fashion from the other two patients. He was offered a basic diet of high caloric liquid mixture[4] plus bread, butter, and jelly, the total containing 95 gm. of protein and 2,500 calories daily beginning on the third day of his metabolic study. Prior to this he had been offered greater amounts of this diet but was unable to tolerate it comfortably. This basic diet was given throughout the period of study. After the first five days of the basic diet he was allowed a limited choice of additional food which consisted of a serving of egg, meat, and a vegetable,

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[1] From the Department of Medicine, State University of New York Medical College at Syracuse, New York.

[2] This study was aided in part by a grant from Parke, Davis & Company, Detroit, Michigan.

[3] Obtained through the courtesy of Dr. Gladys L. Hobby, Charles Pfizer and Co., Brooklyn, New York.

[4] Prepared using Somagen and Lipomul obtained through the courtesy of the Upjohn Company, Kalamazoo, Michigan.

each once daily. These articles of food were allowed after he had consumed the basic liquid diet. The amounts of food ingested were calculated and the nitrogen intake was derived from these calculations. After five days of this limited choice of extra food he was given isoniazid, 2.5 mg. per kg. of weight, for eight days while the same dietary regimen was followed.

Daily urine collections were carried out, using toluol preservative. Stool collections, which were performed by the use of carmine red markers, were collected using benzoic acid in measured amounts for preservation. The nitrogen content of the diet for the first 2 patients and all urine and stools were analyzed by the macro-Kjeldahl method. The patients

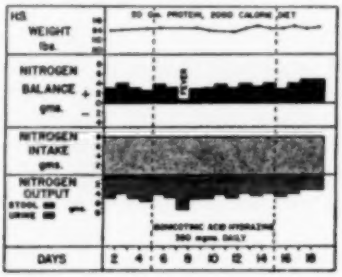

FIG. 1. N. S., convalescing from pneumonia. Positive nitrogen balance unaltered by isoniazid.

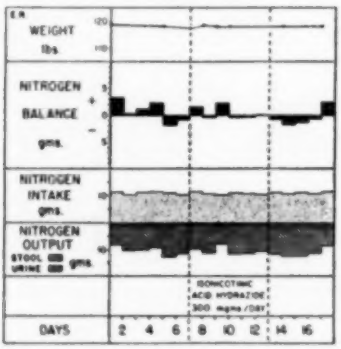

FIG. 2. Chronic hepatitis syndrome. Nitrogen equilibrium unaltered during isoniazid administration.

were weighed frequently and observed carefully for evidence of toxic effects and for evidence of increasing appetite.

RESULTS

Nitrogen balances in patients H. S. and E. R. (figures 1 and 2) were essentially unchanged during the administration of isoniazid. Patient H. S. revealed a slightly positive nitrogen balance throughout his metabolic study and this was not altered by the administration of the agent. Patient E. R. was in nitrogen equilibrium throughout the study and likewise demonstrated no significant

changes. In both patients there was no obvious increase in appetite and neither requested more food during the experimental period. No untoward effects were noted.

In patient G. H., suffering from nontropical sprue, anorexia and malnutrition were significant factors prior to the initiation of the basic liquid diet regimen. On this diet he was able to ingest an adequate amount of protein and calories, but a significant caloric loss occurred in the feces and approximately 30 per cent of the fat ingested was recoverable from his stools. There was a slight increase in the nitrogen and caloric intake when the limited choice of food was allowed in addition to the basic diet, but this did not further increase during the administration of isoniazid and no increased hunger was noted (figure 3). In this patient, positive nitrogen balance was noted throughout the study, a result that was not unexpected in view of his debilitated condition. There was a slight increase in the amount of nitrogen retained during the initial period of limited choice of food but this subsided somewhat during the period of isoniazid administration.

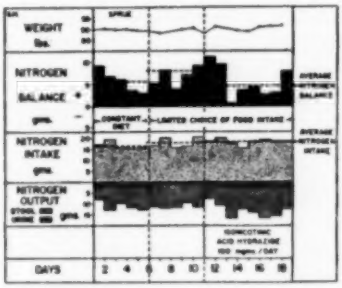

FIG. 3. Nontropical sprue. No change in appetite, food intake, or nitrogen balance attributable to isoniazid.

This decrease in nitrogen retained was, however, probably due to a return toward nitrogen equilibrium in this patient who had been in previous positive nitrogen balance. No change in weight occurred, and stool fat was not altered during the period of isoniazid administration. No untoward effects of the antimicrobial agent were noted.

DISCUSSION

It should be emphasized that the results observed apply only to isoniazid as it has been used in nontuberculous patients for short-term periods. These results indicate that there is no anabolic effect of the drug per se in patients without tuberculosis. There may be, however, an indirect anabolic effect in patients with active tuberculosis who receive this agent which is due to control of the infection, and an anabolic effect even in nontuberculous patients might be noted in longer periods of study. In addition, other hydrazide derivatives of isonicotinic acid may be more potent stimulators of appetite or anabolism. It would appear most likely, nevertheless, that both the anabolic and the appetite-stimulating effects

of these compounds in patients with tuberculosis are due to the control of infection which is afforded by the use of this agent.

It is to be noted that isoniazid differs from the effect of aureomycin which, under similar conditions, has been demonstrated to produce catabolism (2, 3). Neither anabolism nor catabolism can be attributed to isoniazid under the conditions of this study of nontuberculous patients.

SUMMARY

In an attempt to determine whether isoniazid possesses anabolic or appetite-stimulating properties, 3 patients with nontuberculous disease have been studied. In all patients nitrogen balance was determined during administration of this agent, and in one subject food intake was followed during a period of limited diet selection to determine the possible development of increased appetite.

No significant alterations in nitrogen balance, appetite, or food intake occurred during the administration of isoniazid for brief (six to eight days) periods.

The results suggest that there is no anabolic effect of this antimicrobial agent per se in nontuberculous patients. Weight gain in tuberculous patients so treated, therefore, is probably dependent upon control of infection.

SUMARIO

El Metabolismo Nitrogenado y la Ingestión Alimenticia en los Enfermos No Tuberculosos que Reciben Isoniacida

Tratando de determinar si la isoniacida posee propiedades anabólicas o apetitosas, se estudió a tres sujetos con afecciones no tuberculosas. En todos, se determinó el equilibrio del nitrógeno durante la administración de dicha substancia y en uno, se observó la ingestión alimenticia durante un perfodo de limitada selección dietética a fin de descubrir la posible aparición de aumento del apetito.

No hubo alteraciones importantes en el equilibrio del nitrógeno, el apetito o la ingestión alimenticia durante la administración de isoniacida durante breves perfodos de tiempo (seis a ocho dfas).

Los resultados indican que este agente antimicrobiano no ejerce efecto anabólico por sí mismo en los sujetos no tuberculosos. El aumento de peso en los tuberculosos tratados con el mismo se debe probablemente al dominio de la infección.

RESUME

Métabolisme de l'azote et apport alimentaire chez le malade non-tuberculeux recevant l'isoniazide

Dans le but de déterminer si l'isoniazide possède des propriétés anaboliques ou exerce une action stimulante sur l'appétit, trois malades atteints d'affections non-tuberculeuses ont été étudiés. Chez tous ces malades l'équilibre azoté a été déterminé au cours de l'administration de ce médicament; chez un des sujets, l'apport alimentaire a été enregistré pendant une période au cours de laquelle un régime alimentaire sélectionné restreint pour déterminer le développement possible d'une augmentation de l'appétit était donné.

Aucune modification significative de l'équilibre azoté de l'appétit ou de l'ingestion alimentaire ne s'est produite au cours de l'administration de l'isoniazide pendant de courtes périodes (six à huit jours).

Les résultats suggèrent que cet agent antimicrobien est dépourvu d'une action anabolique spécifique chez le malade non-tuberculeux. Donc, le gain de poids qui se manifeste chez

les malades tuberculeux traités par l'isoniazide est probablement le résultat du contrôle de l'infection.

Acknowledgments

The author expresses his gratitude to Mrs. Joan M. Noll for technical assistance; and to Mrs. Clarice Shepard and Miss Phyllis Utter for the diets used in this study.

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DESIGN AND OPERATION OF A LABORATORY FOR EXPERIMENTAL TUBERCULOSIS[1]

MORRIS SOLOTOROVSKY, HARRY J. ROBINSON, AND MICHAEL KNIAZUK

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INTRODUCTION

With the expanding activity in experimental tuberculosis, especially as related to large-scale chemotherapy studies, safety to personnel has become an increasingly important factor in successful laboratory operation. The subject of safety to laboratory personnel is receiving attention as a result of studies on air-borne infection and the increasing number of initially tuberculin-negative personnel available for employment in tuberculosis laboratories. A recent survey of laboratory infections by Sulkin and Pike (1) shows that brucellosis has accounted for the highest number of laboratory infections and that tuberculosis is a close second. Fish and Spendlove (2) have listed a series of rules to be followed in tuberculosis laboratories which covers the use of exhaust hoods for transfer of cultures, use of equipment, handling of animals, and decontamination procedures. They did not discuss laboratory design, complete ventilation, or methods for housing infected animals. The safety problem has also been discussed by Long (3), who emphasized measures to prevent contamination of the air. In this regard he cited the findings of Wells and Lurie (4), indicating that ultraviolet light prevented cross-infection to susceptible animals.

In the present report the writers describe a tuberculosis research unit, a method of housing infected mice, and techniques that are specifically designed to minimize the hazard of both contact and air-borne infections. This description is presented because a number of investigators have found various details of architectural design, the method of housing infected mice, and details of procedure as used in the unit herein described of value in modifying existing laboratories and planning new laboratories. The equipment and procedures are not as elaborate as those used in installations such as that at Camp Detrick (5) and the Naval Medical Research Unit No. 1 (6), which are designed to meet the problem of cloud chamber operations, but appear to be adequate for more conventional procedures.

GENERAL PLAN OF LABORATORY AND ANIMAL ROOMS

Floor Plan and Equipment

The floor plan of the unit is shown in figure 1. The terms "non-hazardous" and "hazardous" in the description which follows are not used in a rigid sense. The laboratory is designed to accommodate experimental infections with high hazard of air-borne infection as compared with tuberculosis. If tubercle bacilli

[1] From the Merck Institute for Therapeutic Research, Rahway, New Jersey.

were being used, viable cultures in unsealed containers would not be kept in "non-hazardous" areas. "Non-hazardous" as here used indicates that culture work is done only intermittently. "Hazardous" indicates that cultures are handled frequently and in relatively large numbers or that infected animals are housed or handled in the area thus designated.

Entrance is effected (figure 1, *1*) into a corridor which permits access to the office (figure 1, *2*), laboratory (figure 1, *3*), or the decontamination rooms (figure 1, *4*, *4A*, *5*, *5A*). Laboratory No. 3 may be designated as a "non-hazardous" area. The decontamination rooms are divided into halves. The one opening into corridor 1 is "non-hazardous" and the adjoining half is "hazardous." The lockers in the "non-hazardous" half are used for street clothes and those in the "hazardous" half for laboratory clothes. The "hazardous" halves of the decontamination rooms open into a corridor (figure 1, *6*), which connects with laboratory 7. Laboratory 7 is used for the performance of *in vitro* tests. It is also used to prepare equipment such as syringes and surgical instruments used in anima

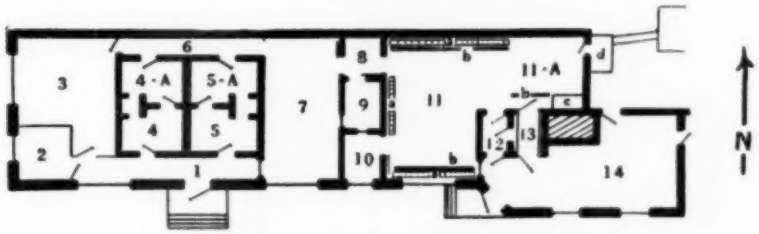

FIG. 1. Floor plan of the tuberculosis unit.

tests. Corridor 8 connects laboratory 7 with the animal room. Two cubicle rooms (figure 1, *9* and *10*) are placed between laboratory 7 and the animal room (figure 1, *11*). One of the cubicle rooms (figure 1, *9*) opens into corridor 8 and the other (figure 1, *10*) opens into the animal room (figure 1, *11*). A recessed area in corridor 8 accommodates a hot air sterilizer and an autoclave.

Animal room: The animal room is divided into an animal rack area (figure 1, *11*) and a service area (figure 1, *11A*). Along the south wall of the service area is a steam room (figure 1, *12*), which also serves as an air lock for transfer of heavy equipment, a large autoclave (figure 1, *13*), and a sink (figure 1, *c*). An incinerator (figure 1, *d*) is situated on the east wall. These heat treatment devices assure that no potentially contaminated material need be removed from the laboratory without heat treatment adequate to destroy the tubercle bacillus. Jars containing infected animals are kept on racks placed along the north and south walls of the animal rack area. Normal animals are kept on racks placed along the east and west walls. Exhaust ducts are placed above the animal racks along the north and south walls. In addition, these areas are surrounded by curtains of ultraviolet light across the fronts and tops of the racks.

At present room 14 is a "non-hazardous" storage area.

Air Supply and Air Removal

Air, conditioned for temperature and humidity, enters each room through ceiling louvers. The path of air flow is from corridor 1 and office to laboratory 3, through corridor 6 to laboratory 7, through corridor 8 to the animal room. The air exhaust of the cubicle rooms is independent of the other areas. The air of the animal room enters through six ceiling louvers located outside the contaminated animal rack areas and is withdrawn through the ducts above the contaminated animal area. The exhaust ducts are connected to a central incinerator stack. Thus, air from the infected animal rack area does not mix with the air supply in the room proper. The air conditioning equipment provides seven to ten changes per hour, and no air is recirculated.

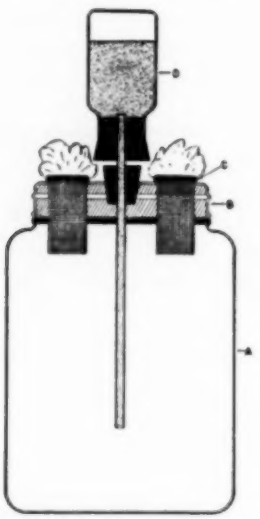

FIG. 2. Jar for housing mice.

Housing of Infected Animals

The method for housing infected mice departs from conventional practice. Groups of four animals each are housed in glass jars (figure 2, *A*), 6 by 6 by 8 inches high with a round opening 4½ inches in diameter. This jar is a commonly used mayonnaise or pickle container. The jars are closed by metal screw-cap lids (figure 2, *B*) pierced by two apertures 1¼ inches in diameter and a third aperture ¾ inch in diameter. The two larger apertures accommodate stainless steel wire mesh wells (figure 2, *C*) 1¾ inches long which are loosely plugged with nonabsorbent cotton. Thus, air passing from the jar is filtered through the cotton. The third opening is closed by a rubber stopper pierced to accommodate the stem of an inverted water bottle (figure 2, *D*). The water bottle is a 50 ml.

specimen bottle. The animals are bedded on bagasse and are transferred to fresh jars twice weekly.

Cubicles and Hoods to Accommodate Infected Animals for Treatment

All manipulations with infected animals and autopsies are performed in hoods located in the two cubicles adjoining the animal room. The hoods (figure 3) are approximately 45 inches wide, 24 inches deep, and 24 inches high. The opening across the front is 7 inches by 45 inches. The units are fabricated of stainless steel with smooth welded construction to facilitate cleaning. The doors (17

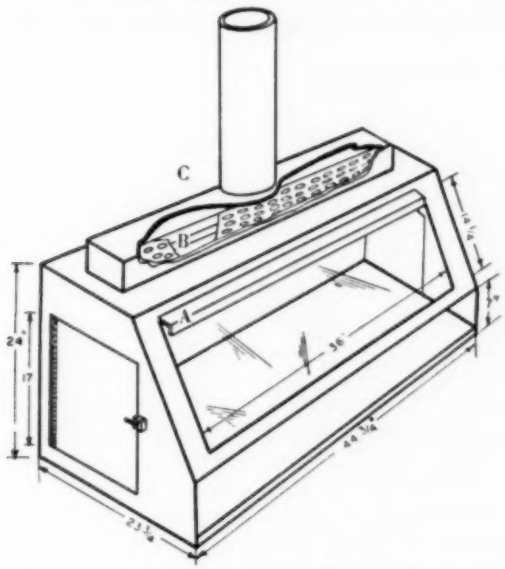

FIG. 3. Operating hood: *A*—fluorescent work light; *B*—ultraviolet sterilizing tube; *C*—exhaust pipe.

inches by 17 inches) are hung on piano-type hinges and are gasketed to obtain tight closure. The hood is irradiated by an ultraviolet light placed at the top of the hood, and working light is supplied by a fluorescent lamp attached to the upper, outer surface. The exhaust duct leaving the hood carries the air to an electrically heated incinerator located in the attic of the building. The incinerator consists of a box 20 inches wide, 16 inches high, and 36 inches long, fabricated from asbestos cement board (trademark "Transite") bolted to an angle-iron frame. It is equipped with 12 pairs of 350-watt strip heating elements, the components of each pair connected in series, the pairs in parallel, across a three-phase 440-volt line. Air passing through the incinerator attains a temperature of approximately 350°C. The incinerated air is then carried to the central in-

cinerator stack. As noted above, the air exhaust of the cubicle rooms is independent of air removal flow in the other rooms of the unit. All air enters through a ceiling louver and is withdrawn by the hood exhaust at a rate of 90 cubic feet per minute. Thus, the movement of air is away from the operator. The door of the cubicle room is covered with stainless steel and is of a sliding self-closing type.

Dress and Cleansing Procedures

The personnel who manipulate animals are dressed in gown, rubber gloves, and surgical masks. In addition, tinted glasses (trademark "Calobar Safety") are worn for protection of eyes from reflected ultraviolet light. Animals are

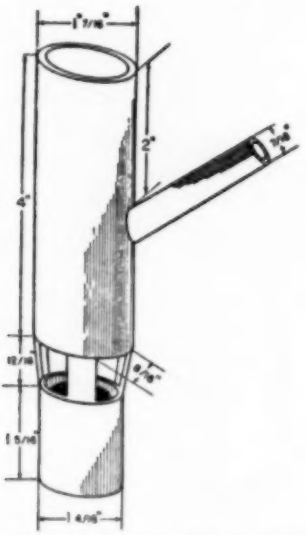

FIG. 4. Chimney for sterilization of platinum inoculating needles.

removed from jars with 12-inch dressing forceps. Dead animals are dipped in 3 per cent coal-tar disinfectant solution (Creolin) before being autopsied. After a cubicle has been used, it is liberally washed with 3 per cent Creolin and hosed with hot water mixed with steam not less than one-half hour after the Creolin wash.

In Vitro Procedures

Nearly all *in vitro* procedures in the laboratory utilize submerged cultures and suspensions which do not require grinding. Technical personnel are trained to avoid breaking the surfaces of liquids, and pipettes containing cultures are never emptied by blowing. A specially designed chimney, shown in figure 4, which is a modification of one used in the Trudeau laboratories, prevents

spattering of infectious material from platinum wires used for the occasional transfer of cultures on solidified media. The chimney is made with all welded construction from brass.

At present a device is being tested which was designed by Bratton (7) for delivery of liquid inoculum into culture tubes which eliminates the potentially hazardous pipetting operation. A 5 ml. pipette mounted vertically on a clamp stand is connected to a 10 ml. syringe mounted horizontally on a second clamp stand by a length of rubber tubing joining the mouthpiece of the pipette with the tip of the syringe. An airtight syringe seal is assured by lubricating the plunger with glycerin. The pipette is filled from the container of inoculum by withdrawal movement of the plunger and inoculum is delivered to culture tubes by forward movement of the plunger.

A system of cubicle operation for *in vitro* manipulations has been described by Youmans (8).

Autopsy Procedures

Personnel are clothed in standard dress for handling infected animals: laboratory trousers, laboratory shoes, gown, mask, rubber gloves, cap, and calobar goggles. Jars containing dead mice or mice which are to be sacrificed are transferred to the hoods. Dead animals are removed from the jars, dipped in 3 per cent Creolin, and then placed on a wire mesh cover of a second pan to drain off excess Creolin solution. Autopsies are performed under the hoods and the desired tissues are placed in cork-stoppered specimen bottles containing preservative. The specimen bottles are immersed in 3 per cent Creolin as they are removed from the hoods and are not touched with bare hands for one hour after contact with the disinfectant. Dead animals and waste tissue are placed in polyethylene plastic bags. The bags are closed and removed to the incinerator. Polyethylene plastic bags have high tensile strength and are watertight. Used instruments such as syringes and surgical tools are placed in stainless steel pans with lids. These pans are transferred to the autoclave after they have been wiped with 3 per cent Creolin. Scraps of bedding or food are dampened with Creolin solution. The hoods are wiped with 3 per cent Creolin and cleaned with hot water or steam no sooner than one hour after application of Creolin solution.

Testing the Air for Contamination

Guinea pigs, initially determined to be tuberculin negative, are kept in open cages among the mouse jars and at various stations in other parts of the areas in which mice are present; there are 6 guinea pig stations. The guinea pigs are tested at three-month intervals for tuberculin reactivity and are autopsied and examined for the presence of tuberculous lesions when they have died. Guinea pigs have survived for an average period longer than one year. To date none of these guinea pigs has shown a tuberculin-positive reaction or evidence of tuberculosis at autopsy. The efficiency of the operating hoods with exhaust in operation is indicated by the inability to recover droplet nucleus sprays of *Serratia marcescens* on plates exposed in the hood and at various levels in the cubicle

room for fifteen-minute periods beginning immediately after ten-minute periods of spraying. The complete removal of droplets is observed in the absence of ultraviolet light. The medical and health program conforms with the one recommended by Fish and Spendlove (2).

SUMMARY

The design and operation of a tuberculosis laboratory planned to minimize danger of laboratory infection is described. The various areas are classified by degree of hazard of infection. Infected animals are housed in a manner to prevent passage of contaminated air into the room. Animals are treated under exhaust hoods in cubicle rooms. The flow of air is from "non-hazardous" areas to "hazardous" areas. Regulations are presented regarding dress, handling of animals for treatment, and performance of autopsies. Guinea pigs exposed in the "hazardous" areas for prolonged periods of time have not acquired tuberculosis. Personnel are carefully trained and indoctrinated, but intelligent, conscientious, and adept performance remains essential for effective use of the equipment and methods.

SUMARIO

Diseño y Funcionamiento de un Laboratorio de Tuberculosis Experimental

Descríbense el diseño y el funcionamiento de un laboratorio de tuberculosis que trata de reducir al mínimo el peligro de las infecciones de laboratorio. Las varias zonas son clasificadas conforme al peligro de infección que encierran. Los animales infectados son alojados de manera que se impida el paso de aire contaminado al aposento, siendo tratados bajo campanas de escape en cubículos. La corriente de aire va de zonas indemnes a zonas peligrosas. Los reglamentos aquí presentados conciernen la ropa, la manipulación de los animales para tratamiento y la ejecución de autopsias. Los cobayos expuestos en las zonas peligrosas durante períodos prolongados de tiempo no han adquirido tuberculosis. El personal está cuidosamente adiestrado y aleccionado, pero para empleo eficaz de las instalaciones y de las técnicas continúa siendo indispensable la labor inteligente, concienzuda y pericial.

RESUME

Projet pour l'établissement et le fonctionnement d'un laboratoire de tuberculose expérimentale

Description d'un projet pour l'établissement et le fonctionnement d'un laboratoire de tuberculose conçu pour réduire au minimum les risques d'infection. Les diverses sections sont réparties selon le degré du risque d'infection. Les animaux infectés sont logés de manière à prévenir le passage de l'air contaminé dans les autres pièces. Les animaux sont traités sous des hottes à échappement d'air dans des compartiments isolés. La circulation d'air se fait des sections non-contaminées vers les sections contaminées. Des règles sur l'habillement du personnel, la manipulation des animaux pour le traitement et l'exécution des autopsies sont présentées. Des cobayes, exposés dans des sections de contamination possible pendant un temps prolongé, n'ont pas contracté la tuberculose. Le personnel est soigneusement entraîné et instruit, mais il est essentiel pour la mise en oeuvre des méthodes et un bon usage de l'équipement que le programme soit exécuté intelligemment et consciencieusement.

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THE SELECTION OF A BOVINE STRAIN OF MYCOBACTERIUM TUBERCULOSIS FOR STUDIES IN EXPERIMENTAL TUBERCULOSIS[1]

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INTRODUCTION

During the past five years numerous papers have appeared describing the use of the mouse for studies in experimental tuberculosis (1–7). The success of such experiments has been found to be greatly influenced by the bacterial strain (5), rate of growth (8), pathogenicity of the strain (9), the age and size of the inoculum (8), the site of inoculation (10), and the strain of the mouse used (5, 6, 10). It has been suggested (5, 6, 11) that bovine strains of *Mycobacterium tuberculosis* give a more uniform death rate than the standard human strain H37Rv.

In an effort to select a more suitable strain for studies in chemotherapy in mice, the present writers have studied under identical conditions the growth rate *in vitro* and pathogenicity in mice of two bovine strains D_4-M_2[2] and D_{114}[3]. In addition, a comparison has been made of the lesions of mice inoculated with the more virulent of the two bovine strains with those in rabbits and guinea pigs.

METHODS

The Strains of Mycobacterium Tuberculosis

The bovine strain D_4-M_2 of *M. tuberculosis* has previously been reported as producing in fourteen days a fatal infection in mice CF No. 1, Tumblebrook and Vanderwerken breeds, when injections were given intravenously with doses in a concentration of 0.1 mg. dry weight of bacteria (6). This strain has been compared in growth rate under identical conditions with strain D_{114}, a biotype of the Ravenel strain.

Culture Media

The two strains were cultured in two different media: one, the *standard medium* used in this laboratory for all chemotherapeutic testing; and a second medium referred to as *formula 11* which differed principally from the *standard medium* in the substitution of enzymatic digest of casein (Difco) in place of vitamin solutions. The two culture media were prepared as follows:

Standard Medium

Part I:

Dibasic sodium phosphate, anhydrous	1.5 gm.
Potassium phosphate	2.0 gm.

[1] From the Laboratory of Pathology and Pharmacology, National Institutes of Health, Public Health Service, Bethesda, Maryland.

[2] Strain D_4-M_2 was kindly supplied by Dr. M. J. Baker of the American Cyanamid Co.

[3] Strain D_{114} was kindly supplied by Dr. D. Hamre of E. R. Squibb & Sons.

Sodium citrate	1.25 gm.
$MgSO_4,7H_2O$	0.3 gm.
Asparagine	2.5 gm.
Sucrose	10.0 gm.
Glycerin	5.0 ml.
Tween 80[4] (10 per cent solution)	1.0 ml.
Water	500.0 ml.

The pH was adjusted to 6.8–7.0 with 40 per cent sodium hydroxide and autoclaved at 15 pounds of pressure for 45 minutes.

Part II: A second part of the culture medium consisted of a solution of vitamins as follows:

Vitamins	*Mg. per 100 ml.*	*Volume ml.*
Niacin	20.0	50.0
Para-aminobenzoic acid	100.0	10.0
Biotin	2.5	40.0
Pyridoxine	100.0	10.0
Thiamin	100.0	10.0
Calcium pantothenate	100.0	10.0
Foric acid	40.0	20.0
Riboflavin	5.0	10.0
		160.0

Each of the vitamin solutions was passed through Seitz filters and combined immediately before using. Ten milliliters of the vitamin mixture were added to each 70 ml. of sterile medium containing Tween 80 (Part I).

Part III: A third part of the medium consisted of serum albumin (Bovine Fraction V—Armour). This was prepared in 2.0 per cent solution and heated to 56°C. for ten minutes, cooled, and sterilized by filtration. Twenty milliliters of this were added to 80 ml. of the base culture medium containing vitamins. No further adjustment of pH was necessary.

Formula 11

This medium differed from the *standard medium* by the addition of Part I of 0.025 gm. of ferric ammonium citrate, 20 ml. of 5 per cent enzymatic digest of casein (Difco), the elimination of additional vitamins as in Part II *standard medium*, and the reduction of the amount of sucrose to 5 gm. Just before using the medium, serum albumin was added to Part I in a proportion of 1 to 4.

Method of Calculating Growth

The two strains D_4-M_3 and D_{114} were cultured for seven months in the two media, in submerged growth, with routine transfers at seven-day intervals. All cultures were carried in quadruplicate, and the mean growth for each strain was obtained from daily turbidity readings[5] from the fourth day to the eighth day. Subculturing was carried out by adding 1 ml. of a seven-day culture to each 9 ml. of fresh medium. Furthermore, the mean growth of all 28 transfers covering the seven-month period was obtained for each strain from the average of the terminal eighth day of growth of each experiment, and the probability error of the standard deviation was calculated.

Pathogenicity of Strains in Mice

The determination of pathogenicity of the two strains was based upon the weight curves, the 50 per cent death point, and histologic examination. Intravenous inoculations of 0.2

[4] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[5] Using Coleman nephelometer and filter No. 8-212.

ml. were made into the dorsal vein of the tail of inbred albino mice (NIH strain). Cultures of 3, 5, and 7 days of age were used to determine which concentration was most suitable for obtaining 50 per cent mortality in two weeks. Estimation of bacilli present in the inoculum was based upon the mean dry weight of 10 ml. of washed cultures after 7 days of incubation. The mean of 10 such weighings gave 0.02 mg. per ml. as an estimate of the concentration of the inoculum. Uniform results in the death of inoculated mice were readily obtained by using 7-day-old cultures.

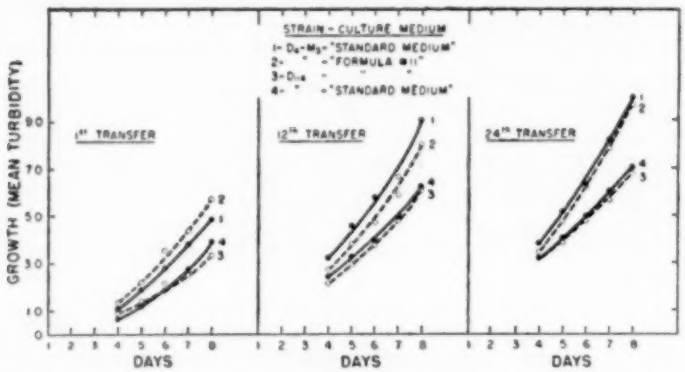

FIG. 1. Comparative rate of growth of strains D_4-M_3 and D_{114} in *standard medium* and *formula 11*.

TABLE 1

STATISTICAL DATA OF GROWTH OF STRAINS D_{114} AND D_4-M_3 ON TWO MEDIA

MEDIA							
Standard				Formula 11			
Strain	Mean	t-Value	Probable Error	Strain	Mean	t-Value	Probable Error
D_4-M_3	9.2	6.66	$P < 0.001$	D_4-M_3	8.2	5.45	$P < 0.001$
D_{114}	6.0			D_{114}	5.8		

RESULTS

Growth Rate of Strains

Comparison of mean growth rates from daily readings during a period of seven months showed the growth of D_4-M_3 to be consistently higher than that of D_{114}. In figure 1 is shown the growth rate during the eight days' experimental period of the first, the twelfth, and the twenty-fourth transfers. Statistical analysis of the data of the growth of these two strains on the two separate media shows the difference to be significant (table 1).

Growth of Strains in Swiss Albino Mice

Virulence of strains D_4-M_3 and D_{114}: It did not necessarily follow that the more rapidly growing strain D_4-M_3 was the more rapidly invasive or pathogenic of the

two. To determine the relative virulence, two experiments were carried out, the first using *seven-day cultures of high concentration of Mycobacteria* in four groups of 18 mice.

Fifty per cent of the mice inoculated with strain D_{114} grown in *formula 11* medium were dead in sixteen days and all were dead in three weeks. Of the mice receiving strain D_{114} in *standard medium*, 61 per cent were dead in seventeen days and all were dead in three weeks. In the mice receiving D_4-M_3 of both groups, 60 per cent were dead on the sixteenth day and 4 were still alive after four weeks; 8 had died during the first nine days after inoculation without showing gross evidence of tuberculosis, although microscopically the tissues were swarming with bacilli. Mean weights in all groups began to fall rapidly after the first week so that it was not possible, using such a heavy concentration, to ascertain which strain had the greatest potency, either on basis of mean weights or mortality.

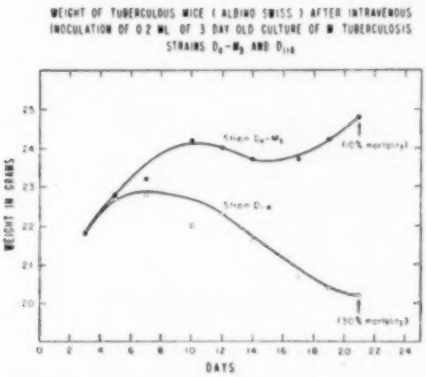
WEIGHT OF TUBERCULOUS MICE (ALBINO SWISS) AFTER INTRAVENOUS
INOCULATION OF 0.2 ML OF 3 DAY OLD CULTURE OF M TUBERCULOSIS
STRAINS D4-M3 AND D114
WEIGHT IN GRAMS
DAYS

FIG. 2. Weight curves of mice after inoculation of 0.2 ml. of a three-day culture of *M. tuberculosis* strains D_4-M_3 and D_{114}.

A second experiment was therefore carried out in which two groups of 20 mice each were inoculated with *three-day cultures of low concentration* of both strains grown in *standard medium*. The weights of the mice inoculated with D_{114} began to fall rapidly in two weeks, while those inoculated with D_4-M_3 rose to a level above those inoculated with D_{114} and tended to maintain that level during a three-week period. Thirty per cent mortality occurred in twenty-one days of those inoculated with D_{114}, while only 10 per cent died of those inoculated with D_4-M_3 in the same time (figure 2).

While *in vitro* studies had shown the slower growth rate of D_{114}, this experiment indicated its greater virulence. Gross lesions of the lungs were evaluated in terms of zero to four-plus. All of the mice inoculated with strain D_{114} showed gross lesions, of which 85 per cent were rated four-plus. Only 50 per cent of the mice inoculated with strain D_4-M_3 showed extensive gross lesions, 35 per cent showed a few scattered lesions, and a few showed none at all. These differences suggested the selection of D_{114} for further studies.

Selection of culture medium: An experiment using 0.2 ml. of seven-day cultures of D_{114} from each of both mediums inoculated into 18 gm. mice yielded no differences in mortality or weight curve. *Formula 11* was used thereafter as it was simpler to prepare.

Selection of age and concentration of culture for inoculation: A fourth experiment was carried out in which eight groups of 18 mice each were used. Cultures of D_{114} grown in *formula 11* of one, three, five, and seven days of age were inoculated in volumes of 0.2 ml. intravenously. In the first series, the inoculum was undiluted, while in the second series the cultures of bacilli were diluted 50 per cent with fresh culture medium. One- and three-day-old cultures were too diluted to cause 50 per cent mortality in two weeks (table 2). All of the mice in the group inoculated with 0.2 ml. of a seven-day-old culture showed extensive lesions, however, while those receiving more dilute cultures showed fewer nodules in the lungs. Only 4 mice in the entire experiment showed no lesions in the lungs.

TABLE 2

EFFECT OF DIFFERENT AGE CULTURES (STRAIN D_{114}) UPON DEVELOPMENT OF DISEASE

SERIES 1—UNDILUTED INOCULUM				SERIES 2—INOCULUM DILUTED 50 PER CENT			
Age of Culture in Days	Number of Mice	Days Required for 50 per cent Mortality	Per Cent with Extensive Tubercles in Lungs	Age of Culture in Days	Number of Mice	Days Required for 50 per cent Mortality	Per Cent with Extensive Tubercles in Lungs
1	18	23	83	1	18	26	77
3	18	22	89	3	18	21	61
5	18	16	89	5	18	19	94
7	18	14	100	7	18	15	89

The data suggest that 0.2 ml. of a seven-day culture gives the desired mortality curves for chemotherapeutic studies.

Pathogenicity of Strains D_4-M_2 and D_{114}

Mice: The rapid loss in weight and precipitous death rate of mice (Swiss strain) inoculated with 0.2 ml. of a seven-day culture of strain D_{114} are shown in figure 3. Fifty per cent died between the fourteenth and sixteenth days; the total period during which deaths occurred was eight days from the first death. All showed gross lesions in the lung at the time of death. This death rate is in sharp contrast to the mouse infection produced by tubercle bacilli of the human strain H37Rv, in which deaths do not occur before three to four weeks after inoculation and continue thereafter for six weeks with some mice surviving for even longer periods (7).

Microscopically, the livers of mice which died early after inoculation with strain D_4-M_3 showed widespread dilation of the sinuses and compression of cords of liver cells by homogeneous fibrin clots in which were varying numbers of scattered cells and numerous acid-fast bacilli. Tubercle bacilli were also abundant in Kupffer cells and in minute sinusoidal groups of phagocytic mononuclear cells.

A few small scattered areas of necrosis were present. The presence of numerous extracellular bacilli suggested active extracellular proliferation of the organisms within the sinuses. The process appeared to have spread by the hematogenous route. Similar lesions were present in the lungs, and occasionally widespread thrombosis of pulmonary arteries and arterioles occurred. Here also extracellular bacilli accounted for a large portion of the bacilli present. In the sinusoids of the spleen a comparable picture was seen.

A similar microscopic picture was observed in the tissues of mice inoculated with the same concentration of bacilli of the D_{114} strain. The principal difference was the onset of the histopathologic changes which required from twelve to fourteen days, at which latter time tubercles in the lungs became grossly visible. The evolution of the infection in the lung was readily deduced from numerous histologic examinations. Small tuberculous foci developed in the septa between alveoli, with a minimum of cellular response. The extension of the foci into alveoli

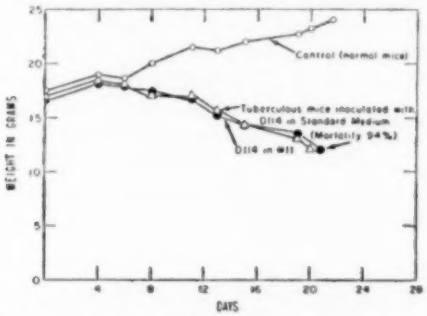

FIG. 3. Weights and mortality of mice inoculated with 0.2 ml. of a seven-day culture of strain D_{114}.

began on approximately the twelfth day and became extensive by the sixteenth day. The development of small numbers of bacilli in the alveolar spaces was rapidly followed by transudation of fluids and exudation of cells into the air sacs, thus leading to the formation of pneumonic foci. The foci enlarged with great rapidity and formed large areas of tuberculous pneumonia within forty-eight hours after the extension into the alveoli. The characteristic lung lesion of mice which die twelve to sixteen days after inoculation is thus an exudative tuberculous pneumonia with multiple lesions.

Microscopically, there appears to be little difference in the basic nature of the disease as produced by strains D_4-M_2 and D_{114}. Differences are related to the time of onset of the disease and subsequent death. With strain D_4-M_2, the more rapidly growing, early death may be caused by acute bacteremia, since gross lesions may not appear although tubercle bacilli are plentiful in the tissues. When death is delayed a few days, the characteristic gross lesions of the lungs appear. These were present in all mice inoculated with D_{114}.

Guinea pigs: Thirty-six guinea pigs were inoculated intraperitoneally with

0.172 mg. of tubercle bacilli strain D_{114}. Fifty per cent of these animals were dead on the sixteenth day, 90 per cent on the twenty-first day (figure 4).

All animals had large encapsulated omental lesions, saturated with bacilli, with firm adhesions to the stomach, liver, and spleen. Lymph nodes were included in some of these masses, which measured 1.5 cm. or more in greatest diameter. Although they might have contributed in some degree to the illness of the animal, it is believed that they were not significant as a direct cause of death. There was no general peritonitis. The spleens contained numerous small tubercles of epithelioid cells containing numerous bacilli. At fourteen days after inoculation these showed no necrosis, but at 16 days or longer most of the tubercles showed coagulation necrosis. Similarly, numerous small lesions with plentiful bacilli appeared in the livers. Many very small foci were identified as arising in Kupffer cells within sinusoids. Again, there was no necrosis at fourteen to fifteen days, whereas,

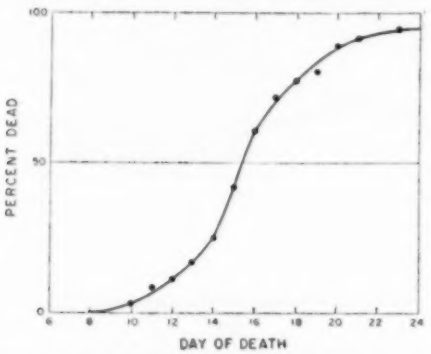

FIG. 4. Death rate of guinea pigs inoculated with strain D_{114}.

on the sixteenth day and later, necrosis of tubercles was the rule. Scattered single bacilli in Kupffer cells were readily found in many animals.

During the fourteen days after inoculation, few lesions were found in the lungs, and in several animals neither lesions nor bacilli were found. At sixteen days, however, all animals showed small septal foci with a few bacilli in each. These ruptured into the alveolar spaces, producing small pneumonic foci which enlarged rapidly. Animals which died on the eighteenth day showed well-developed pulmonary lesions but these were insufficient in extent to account for the death rate of two-thirds of the animals which occurred during the third week. It is suggested that the development of necrosis in a majority of foci on or about the sixteenth day coincides with the known time of appearance of hypersensitivity in guinea pigs infected with tuberculosis (12*b*) and that a toxic reaction from this may be an additional factor in the mortality rate.

Rabbits: Five rabbits inoculated intravenously with 0.172 mg. of tubercle bacilli of strain D_{114} died on the twelfth, fourteenth, fifteenth, sixteenth, and twenty-seventh days, respectively. The lesions of the animal which died on the

twenty-seventh day were identical with those of rabbits which died at the same time from similar infection with a standard Ravenel bovine strain. The animals which died earlier showed much heavier infection of the livers than of the lungs, with numerous small foci suggestive of an infiltrating process, the foci often being contiguous and widely distributed within sinusoids. Miliary foci in the lungs were plentiful, and bacilli were common in all lesions. The first 4 animals to die showed a serous peritoneal transudate in small quantity but no true peritonitis.

Rats proved refractory to infection with strain D_{114} within the limits of the investigation.

DISCUSSION

The most interesting question raised by these two strains of bovine tubercle bacilli is their potential biologic significance. A bovine tubercle bacillus D_{114} which killed guinea pigs in sixteen days was something new and startling. Likewise, the death (presumably with bacteremia) from tuberculosis in eight days in mice (D_4-M_3), although not a uniform occurrence, suggests that the field of pathogenicity of strains of tubercle bacilli is still well open to further exploration.

For chemotherapeutic studies, strains of organisms are required which do not fluctuate in virulence and do produce consistent results over long periods of time. Strain D_{114} has shown great uniformity in every cultural respect. In addition, it has produced excellent early uniform death rates in all experiments, with abundance of gross and microscopic lesions. It appears to be well suited as a test organism.

SUMMARY

A comparative study of the growth rate in submerged culture of the bovine strain D_4-M_3 and the biotype of the Ravenel strain, D_{114}, in two different media has been performed. It was found that the growth of strain D_4-M_3 is consistently more rapid than D_{114}, whereas D_{114} is the more virulent in mice and possesses a high virulence for guinea pigs and rabbits. Strain D_{114} appears to be most suitable for chemotherapeutic studies.

SUMARIO

La Selección de una Cepa Bovina del Mycobacterium Tuberculosis para Estudios de la Tuberculosis Experimental

Un estudio comparativo de la velocidad del desarrollo en cultivo sumergido de la cepa bovina D_4-M_3 y del biotipo de la cepa Ravenel, D_{114}, en dos medios distintos demostró que el crecimiento de la cepa D_4-M_3 es constantemente más rápido que el del D_{114} ,en tanto que éste último es el más virulento en los ratones y posee mucha virulencia para los cobayos y los conejos. La cepa D_{114} parece prestarse mejor para estudios quimioterapéuticos.

RESUME

La sélection d'une souche bovine de Myocbactérium tuberculosis pour l'étude de la tuberculose expérimentale

Une étude comparative de la rapidité de la croissance d'une culture submergée d'une souche bovine D_4-M_3 et du biotype de la souche Ravenel, D_{114}, dans deux milieux différents

a montré que le développement de la souche D_4-M_3 est constamment plus rapide que celle de D_{114}; d'autre part, D_{114} se montre plus virulente chez la souris, elle possède une virulence très marquée chez le cobaye et le lapin. C'est la souche D_{114} qui semble convenir le mieux aux études chimiothérapeutiques.

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A COMPARATIVE STUDY OF STREPTOMYCIN AND DIHYDROSTREPTOMYCIN IN PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

The relative virtues and shortcomings of streptomycin and of dihydrostreptomycin in the therapy of tuberculosis have been debated frequently and at length, although not conclusively. The need for a controlled clinical appraisal of these two drugs became so apparent that it led to the organization (late in 1950) of a cooperative study in eight tuberculosis hospitals. Four of these were situated in New York State and four in other parts of the country.

In this report will be found the collated experience in the latter group. The data from the New York institutions are presented in the article immediately following this one.

As originally planned, the objectives of the study were to compare highly purified, potent preparations of streptomycin[2] and dihydrostreptomycin[2] with respect to therapeutic efficacy, toxicity, and influence on the emergence of drug-resistant strains of tubercle bacilli. Similar numbers of patients with comparable types of tuberculous pulmonary involvement were to be treated with each drug under as closely identical conditions as possible.

PLAN OF INVESTIGATION

A protocol governing all participating hospitals was drawn up, jointly approved, and observed throughout. The following basic requisites were followed:

All patients with pulmonary tuberculosis who were judged to be in need of at least four months' treatment with streptomycin and para-aminosalicylic acid (PAS) were included in the series. The only exclusions were patients known to have received two or more weeks of such chemotherapy previously. Patients with such complications as tuberculous laryngitis, tracheobronchitis, or enteritis were included in the study. Cases of wholly extrapulmonary tuberculosis, however, were excluded. Auxiliary collapse measures or surgical procedures were allowable at any juncture when indicated.

Patients were alternately assigned to receive material "A" or material "B" (crystalline dihydrostreptomycin sulfate or streptomycin sulfate, respectively). The manufacturer supplied vials of these drugs equipped with double labels. The perforated outer label, correctly identifying the drug during shipment, was removed before the material reached its point of employment. This precaution was taken so that a reasonably "blind" test would be achieved and so that medical staffs, both when nominating patients for treatment and

[1] From the Glen Lake Sanatorium, Oak Terrace, Minnesota; North Carolina Sanatorium for the Treatment of Tuberculosis, McCain, North Carolina; Chicago Municipal Sanitarium, Chicago, Illinois; and Weimar Joint Sanatorium, Weimar, California.

[2] Supplied through the courtesy of Merck & Co., Inc., Rahway, New Jersey.

when evaluating the results, would not be swayed by knowledge of which drug was implicated.

Treatment consisted of all traditional hospital measures plus chemotherapy administered on a daily basis. Patients received 1 gm. of streptomycin or dihydrostreptomycin in a single intramuscular injection per day plus 12 gm. of PAS, given orally in divided doses. Patients repeatedly unable to tolerate at least 8 gm. of PAS per day were eliminated from the study. In one hospital a small number of patients received the streptomycin or dihydrostreptomycin in a dosage of 1 gm. every third day but were given PAS in the usual amount daily.

The standard duration of treatment was 120 consecutive days plus or minus one week. Patients could be carried beyond this point if, in the opinion of the physicians, chemotherapy should not be interrupted at four months.

At the time that each patient was assigned to the study, medical staffs established, for future reference, one or more therapeutic objectives. At the conclusion of treatment the degree of success or failure in achieving these objectives was determined and recorded.

Follow-up observations were continued for a minimum of six months in every case before a patient could qualify for inclusion in the reportable group. Prior to entering the study and periodically thereafter, a complete bacteriologic examination of the sputum was performed for each patient. Routine laboratory tests of blood and urine were done, the patient was weighed, and one or more satisfactory chest roentgenograms were made within one week prior to the beginning of treatment. These were compared with subsequent films obtained at appropriate intervals.

Observations for vestibular and auditory toxicity included initial and repetitive questioning for symptoms of disturbed vestibular or auditory function, demonstration (whenever feasible) of ability to walk a chalk line, caloric tests (when facilities were available), and careful audiometric charting. This last-named procedure was conducted prior to therapy, at the conclusion of 120 days of treatment, and approximately six months a fter treatment. Every effort was made to have the audiometric testing done by one or two competent persons in a given hospital under satisfactory test conditions. Duplicate audiometer charts were submitted to a central otologic authority for checking of interpretations made locally in each hospital.

OBSERVATIONS

In the four hospitals, 384 patients were placed in the cooperative study. Of these, 62 were dropped for various reasons during the basic 120-day period, as listed below:

Dead from tuberculosis	9
Misdiagnosed	3
Coexisting renal impairment found	1
Discharged, transferred, or left contrary to medical advice	27
Developed allergy or toxicity to drugs	22

During the six months of post-treatment observations, an additional 28 persons were lost to clinical or statistical analysis. Accordingly, the finally reportable group numbers 294 patients.

Classification of Tuberculosis

Of the 294 patients who completed the study according to the plan, 145 were treated with streptomycin and 149 with dihydrostreptomycin (table 1). Analysis of their clinical status at the outset of chemotherapy reveals that relatively few

cases of minimal pulmonary tuberculosis were included, there being 11 (7.6 per cent) in the streptomycin group and 6 (4.0 per cent) in the dihydrostreptomycin group. Moderately advanced disease was present in 36 patients (24.8 per cent) who received streptomycin and in 43 (28.9 per cent) who were given dihydrostreptomycin. In the far advanced category were 98 (67.6 per cent) and 100 patients (67.1 per cent), respectively. It is thus notable that the over-all distribution was remarkably comparable.

TABLE 1

EXTENT OF DISEASE AT START OF CHEMOTHERAPY

DRUG	NUMBER OF PATIENTS RECEIVING THERAPY 120 DAYS	PULMONARY LESIONS RATED		
		Minimal	Moderately Advanced	Far Advanced
Streptomycin	145	11	36	98
Dihydrostreptomycin	149	6	43	100

TABLE 2

ACHIEVEMENT OF THERAPEUTIC OBJECTIVE

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	DEGREE OF SUCCESS NOTED			
		Complete as Hoped for	Partial but Significant	Slight Only	Failure
Streptomycin	145	73	47	19	6
		82.8 per cent			
Dihydrostreptomycin	149	81	40	21	7
		81.2 per cent			

Comparative Therapeutic Efficacy of Streptomycin and Dihydrostreptomycin

Attainment of objectives: As a measure of therapeutic effectiveness, the comparison of the achievement of treatment objectives by means of either drug (table 2) is of interest. Definitely favorable results were recorded at the 120-day point in 120 patients (82.8 per cent) treated with streptomycin and in 121 patients (81.2 per cent) who received dihydrostreptomycin. These totals are arrived at in each instance by adding those cases in which achievement was judged complete to those in which partial but gratifying results were observed. Likewise, by combining the figures representing merely slight success with those for outright failure, there were 25 patients (17.2 per cent) in the streptomycin group and 28 (18.8 per cent) in the group which received dihydrostreptomycin.

Roentgenographic course: A second useful yardstick of efficacy was the evaluation of improvement as revealed by serial roentgenograms. In table 3 it may be seen that 131 patients (90.3 per cent) who had been treated with streptomycin

showed significant roentgenographic improvement after four months, while for those treated with dihydrostreptomycin the figure was 130 (87.2 per cent). In each drug group the percentage showing no roentgenographic change or actual worsening is so small as to render attempts at comparison unjustifiable. Nor would it be defensible to try to explain the few additional failures in the dihydrostreptomycin column by the fact that the group on this drug at the outset contained fewer minimal cases and was slightly more weighted with cases of moderately advanced disease.

Bacteriologic findings: Sputum "conversions" at the end of 120 days of chemotherapy are summarized in table 4. Prior to treatment, 134 patients (92.4 per cent) placed on streptomycin had sputum positive for tubercle bacilli. For

TABLE 3

ROENTGENOGRAPHIC IMPROVEMENT

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	ROENTGENOGRAPHIC CHANGES OBSERVED		
		Better	No Change	Worse
Streptomycin	145	131 (90.3 per cent)	10	4
Dihydrostreptomycin	149	130 (87.2 per cent)	10	9

TABLE 4

BACTERIOLOGIC FINDINGS

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	SPUTUM FINDINGS			
		Pretreatment		At 120 Days	
		Positive (All Methods)	Negative	Positive (All Methods)	Negative
Streptomycin	145	134 (92.4 per cent)	11	40 (27.6 per cent)	105
Dihydrostreptomycin	149	134 (89.9 per cent)	15	57 (38.2 per cent)	92

the dihydrostreptomycin group, the patients with sputum positive for tubercle bacilli at the outset also numbered 134 (89.9 per cent). After four months of treatment, all methods of examination succeeded in confirming the presence of positive bacteriologic findings in 40 patients (27.6 per cent) who had received streptomycin and in 57 persons (38.2 per cent) who had been on dihydrostreptomycin.

In the patients whose sputum continued to be positive for tubercle bacilli, so few instances were encountered of drug-resistant strains that this problem was of insignificant proportions. No demonstrable difference between the two drugs in this respect was apparent.

Thus, with the one possible exception of percentage of sputum "conversions" (table 4) and subsequent maintenance of negative bacteriologic findings, where streptomycin seemed to excel slightly, it is apparent that no statistical superiority of one drug over the other was established in this study. To all intents and purposes, and in most respects studied, their relative effectiveness in treatment proved equivalent. This was true whether daily or intermittent therapy was

employed. Differences in the symptomatic response to either drug with respect to abolition of fever, diminution in frequency and severity of cough, reduction in sputum volume, and gain in weight were not remarkable.

Toxicity

Allergic or toxic manifestations occurred in a considerable proportion of patients. However, these phenomena were of sufficient severity to demand the cessation of chemotherapy in only 22 persons.

Reference to table 5 will show that major intolerance to PAS (usually gastrointestinal) made it necessary to remove only 5 patients from the study. (By chance, these 5 persons were all in the streptomycin group.) Severe allergic responses to the drug employed (possibly complicated by concomitant PAS intolerance in a few instances) called for the discontinuance of treatment of 12 patients who received streptomycin and of 2 who received dihydrostreptomycin. In no instance was it found advisable to interrupt treatment because of signifi-

TABLE 5

PATIENTS REMOVED FROM STUDY BECAUSE OF DRUG TOXICITY

DRUG	NUMBER OF PATIENTS STARTED ON THERAPY	TYPE OF TOXICITY			
		Intolerance to PAS	Allergic Response	Ototoxic Changes	
				Vestibular	Auditory
Streptomycin	195	5	12	2	0
Dihydrostreptomycin	189	0	2	1	0

cant auditory toxicity, but in 2 patients on streptomycin and in one on dihydrostreptomycin such interference was deemed essential because of severe vestibular disturbances.

The staff of one hospital reported a number of extraordinarily severe drug reactions, necessitating cessation of streptomycin and PAS in 9 patients, 5 of whom had shown grave systemic responses. All of the 5 who suffered serious systemic reactions were Negro patients, and the difficulty developed during the fifth week of treatment.

> One of these patients, a 15-year-old girl, developed exfoliative dermatitis, a leukocyte count of 69,000 per cu. mm., and had anuria for eighty-four hours. Under suitable emergency treatment she survived without evidence of permanent damage to kidneys, liver, or hemopoietic system. However, intracutaneous skin tests to streptomycin and to PAS remained positive four months after the acute episode. Bullae appeared at the test sites, with the threat of spreading dermatitis in the surrounding areas and with the reappearance of abnormal findings, erythrocytes and casts, in the urine.

In 2 other patients of the 5 in this group from this one hospital, skin sensitivity, although not so violent as in the patient reported above, remained similarly demonstrable for several months.

Generalized rash, accompanied by a sharp rise in temperature, albuminuria, hyaline and granular casts in the urine, and slight elevation of the blood nonprotein nitrogen, occurred in 3 Negro men. One of these had a leukocyte count of

22,000 per cu. mm., with 16 per cent eosinophils. In the other 2, leukopenia without eosinophilia was present temporarily.

The same type of reaction just described occurred in the fifth Negro patient, a woman. Further, she experienced paresthesias and muscular twitching. Her leukocyte count for a time dropped below 4,000 per cu. mm. without evidence of eosinophilia.

In all these instances, symptoms subsided promptly with the discontinuance of chemotherapy and the institution of symptomatic measures. In 3 Negro men and 2 other patients in whom treatment with streptomycin had to be stopped, it was found possible to continue therapy (outside the confines of the study, however) by administering dihydrostreptomycin on a twice-a-week regimen.

Ototoxicity

Vestibular dysfunction: Vestibular involvement occurred preponderantly among the patients who received streptomycin (table 6). It will be seen that, in the 63

TABLE 6

INCIDENCE AND SEVERITY OF VESTIBULAR DISTURBANCES

DRUG	NUMBER OF PATIENTS STARTED ON THERAPY	NUMBER OF PATIENTS WITH VESTIBULAR TOXICITY			NUMBER OF PATIENTS REMOVED FROM STUDY BECAUSE OF VESTIBULAR TOXICITY
		Slight (Vertigo)	Moderate (Ataxia)	Severe(Non-reacting Labyrinth)	
Streptomycin	195	31	10	6	2
Dihydrostreptomycin	189	14	2	0	1

persons who experienced vestibular difficulty, the manifestations were generally slight and were mainly marked by the subjective complaint of vertigo. There were 31 instances of this reaction with streptomycin and 14 with dihydrostreptomycin. Ten patients on streptomycin and 2 on dihydrostreptomycin suffered moderate vestibular damage, characterized by ataxia of less than disabling degree. The 6 reported instances of severe vestibular disturbance, i.e., reactions in persons who suffered disabling ataxia or developed a nonreacting labyrinth, all occurred in streptomycin-treated persons.

Auditory impairment: Analysis of the audiometric and clinical reports on all of the patients (table 7) reveals a significant incidence of auditory toxicity. In all, 81 instances of auditory impairment of varying degree were recorded; 60 in patients treated with dihydrostreptomycin and 21 in those who received streptomycin.

It should be noted that in the majority of patients the loss in hearing acuity was so mild as to be unknown to the patient and became apparent only on audiometric testing or was suspected when complaint of sustained tinnitus occurred. There were 38 persons in this subclinical category in the dihydrostreptomycin group and 10 among the streptomycin-treated patients.

Clinical loss of hearing was scored on the basis of Fletcher's criterion, which determines the losses as shown by the audiogram at 500, 1,000, and 2,000 cycles

per second and then uses the average of the two smallest losses. According to Fletcher's rule, therefore, slight hearing loss (5 to 20 decibels) was discovered in 18 patients in the dihydrostreptomycin group and in 11 patients in the streptomycin group. Instances of moderate clinical deafness (loss of 25 to 40 decibels) occurred in 4 patients on dihydrostreptomycin; in none, on streptomycin. No findings were encountered to suggest a severe degree of hearing loss (45 decibels or more) in any patient on either of the two streptomycins.

TABLE 7

INCIDENCE AND SEVERITY OF AUDITORY DISTURBANCES

DRUG	NUMBER OF PATIENTS STARTED ON THERAPY	NUMBER OF PATIENTS WITH AUDITORY TOXICITY				NUMBER OF PATIENTS REMOVED FROM STUDY BECAUSE OF AUDITORY TOXICITY
		Subclinical Hearing Loss	Clinical Hearing Loss			
		Audiometric and/or Tinnitus Only	Slight: (5 to 20 db)	Moderate: (25 to 40 db)	Severe: (45 db and over)	
Streptomycin	195	10	11	0	0	0
Dihydrostreptomycin	189	38	18	4	0	0

TABLE 8

ONSET OF OTOTOXICITY

FIRST NOTED	DAYS AFTER STARTING THERAPY	TYPE OF OTOTOXICITY AND THE DRUG RESPONSIBLE			
		Vestibular		Auditory	
		Streptomycin	Dihydrostreptomycin	Streptomycin	Dihydrostreptomycin
During therapy	0–60	34	7	6	8*
	61–120	12	8	7	16
Post-treatment	121–300	1	1	8	36
Total instances		47	16	21	60

* Tinnitus only: All reported from one hospital.

With respect to the time of onset of ototoxicity, it is revealed in table 8 that vestibular damage was more prone to make itself evident during the first half of the 120-day treatment period, particularly when streptomycin was the drug employed. Nevertheless, vestibular impairment could and did occur later and appeared in one instance with each drug long after the conclusion of therapy. Many of the patients with vestibular signs or symptoms showed excellent compensation or complete reversibility of the phenomena relatively soon after therapy was discontinued.

Conversely, auditory damage, of higher incidence with dihydrostreptomycin than with streptomycin treatment, tended to show up late in therapy and, even more frequently, some time after treatment had stopped. In at least one case, the impairment was not apparent until five months beyond the 120-day observa-

tion point. In a few instances a moderate progression of the hearing loss was detectable by serial audiograms. Thus far, no evidence has accumulated to suggest that auditory loss is reversible.

COMMENT

In this study, in order to compress the investigation into a relatively short but meaningful period of time, daily administration of the streptomycins was employed. However, the body of experience which has been accumulated in the institutions of the present writers and elsewhere would suggest that, in all but the most acute cases of tuberculosis, for which daily treatment may be mandatory, regimens of intermittent dosage are adequate to produce a satisfactory therapeutic response. Regimens of intermittent therapy subject the patient to a smaller quantity of the drug for a given period of time while, at the same time, they reduce the risk of the emergence of drug-resistant infections. With such intermittent therapy, the likelihood of the development of ototoxicity, particularly of the auditory variety, can be minimized or appreciably postponed.

SUMMARY

Both streptomycin and dihydrostreptomycin were shown to be valuable agents in the chemotherapy of amenable forms of tuberculosis in properly selected patients.

It was not possible in the present study to determine any significant difference in the therapeutic efficacy of these two drugs.

When administered in combination with para-aminosalicylic acid (or one of its salts), neither form of streptomycin was associated with the emergence of a significant incidence of drug-resistant infections.

Vestibular toxicity, which generally occurred relatively early in the course of treatment, was more frequently encountered with streptomycin.

Auditory toxicity, which tended to occur relatively later, was more frequently due to dihydrostreptomycin. Although usually ranging from subclinical to moderate in degree, the auditory disturbance can be of serious proportions. It can be progressive or may appear belatedly after chemotherapy has ceased. High tone (audiometric) loss or complaint of sustained high-pitched tinnitus may represent the first indication of oncoming trouble and should always be considered significant.

Allergic responses to streptomycin were not infrequent and were occasionally severe. When dihydrostreptomycin was employed, however, allergic reactions were rarely encountered.

SUMARIO

Estudio Comparado de la Estreptomicina y la Dihidroestreptomicina en la Tuberculosis Pulmonar

Tanto la estreptomicina como la dihidroestreptomicina resultaron ser agentes valiosos en la quimioterapia de las formas susceptibles de tuberculosis en enfermos debidamente escogidos.

En el estudio actual, no fué posible descubrir la menor diferencia importante en la eficacia terapéutica de dichos dos antibióticos.

Administrados en combinación con el ácido para-aminosalicílico (o una de sus sales), ni

uno ni otro antibiótico produjo más que formación ocasional de resistencia microbiana de alguna importancia.

La toxicidad para el vestíbulo, que en general aparece relativamente temprano en el transcurso del tratamiento, fué observada más frecuentemente con la estreptomicina.

La intoxicación auditiva, que solió observarse relativamente más tarde, se debió más a menudo a la dihidroestreptomicina. Aunque variando habitualmente de subclínica a moderada, puede alcanzar proporciones graves, y ser progresiva, o aparecer tardíamente después de la quimioterapia. La pérdida (audiométrica) de los tonos altos o la queja de zumbidos de oídos agudos y sostenidos puede representar la primera señal de trastorno inminente y deben ser atendidas siempre.

Las reacciones alérgicas a la estreptomicina no fueron raras y a veces fueron graves. Sin embargo, al emplear la dihidroestreptomicina, fueron observadas con mucha rareza.

RESUME

Étude comparative de la streptomycine et de la dihydrostreptomycine dans la tuberculose pulmonaire

La streptomycine et la dihydrostreptomycine se sont montrées toutes deux des agents de valeur dans les formes de tuberculose sensibles à la chimiothérapie chez des malades soigneusement sélectionnés.

Cette étude n'a pas permis de déterminer une différence significative quelconque dans l'efficacité thérapeutique de ces deux antibiotiques.

Quand ces antibiotiques ont été administrés en association avec l'acide para-aminosalicylique (ou un de ses sels) ce n'est qu'exceptionnellement qu'une résistance des germes à l'un ou à l'autre s'est manifestée de façon significative.

La toxicité vestibulaire qui se manifestait généralement à une période relativement précoce du traitement, était plus fréquemment rencontrée avec la streptomycine.

La toxicité auditive tendant à se manifester relativement plus tard, était plus fréquemment provoquée par la dihydrostreptomycine. Habituellement cette toxicité était d'un degré variant entre subclinique et modéré, toutefois elle peut atteindre des proportions importantes; elle peut être progressive ou se manifester tardivement après la cessation de la chimiothérapie. La perte des sons d'intensité élevée (audiométriques) ou la présence de bourdonnements continus de sons d'intensité élevée peuvent représenter les signes avant-coureurs d'une lésion et il y a lieu de les prendre en considération.

Les réactions allergiques à la streptomycine n'étaient pas rares, elles se sont montrées parfois sévères. D'autre part, quand la dihydrostreptomycine était utilisée, les réactions allergiques étaieut rarement observées.

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A COMPARATIVE STUDY OF STREPTOMYCIN AND DIHYDROSTREPTOMYCIN IN PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

In an effort to determine possible points of difference in the usefulness and safety of streptomycin and dihydrostreptomycin in the treatment of tuberculosis, a cooperative study of the two drugs was started in eight hospitals throughout the United States late in 1950. The present report summarizes the results obtained in four participating New York State tuberculosis hospitals. In these institutions the provisions of a standard protocol which was followed in all of the eight hospitals were generally observed.

PLAN OF INVESTIGATION

All newly admitted patients with pulmonary tuberculosis judged to be in need of at least 120 consecutive days of chemotherapy were randomized alternately into the streptomycin or the dihydrostreptomycin group. Almost all were adults, and the aim was to include only patients who had not previously undergone treatment with either drug.

Two modifications of the general plan were permitted to conform with practices then being followed in the New York State tuberculosis hospitals and to conform with another investigation then in progress. These were:

1. The dosage of streptomycin[2] or dihydrostreptomycin[2] was 20 mg. per kg. of body weight per day, rather than the 1 gm. dose per day used in the other four hospitals.
2. Treatment could be continued beyond the basic 120-day period whenever such extension of therapy was deemed advisable by the medical staff of the hospital concerned. This represented an extension of the cooperative study herein reported to become part of a special investigation of prolonged drug therapy in a larger number of patients in the New York State tuberculosis hospitals.

The patients included in the present report, therefore, consist of two groups: a small number, who received only the basic 120 consecutive days of chemotherapy (plus or minus seven days), and the vast majority, who received drug therapy for longer periods. The special protocol governing extension of treatment provided that increments beyond the 120-day point would be in multiples of two months each. Decision to continue therapy was based upon the degree of attainment of certain prestated objectives for which chemotherapy had been initiated.

Patients were excluded from clinical analysis if drug therapy was omitted for any reason

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[1] From the New York State Department of Health, Albany, New York; Mount Morris Tuberculosis Hospital, Mount Morris, New York; Ray Brook State Tuberculosis Hospital, Ray Brook, New York; Homer Folks Tuberculosis Hospital, Oneonta, New York; and Hermann M. Biggs Memorial Hospital, Ithaca, New York.

[2] Supplied through the courtesy of Merck & Co., Inc., Rahway, New Jersey.

for fourteen or more consecutive days. Likewise, they were not included in the final analysis if the period of "desensitization" following development of drug allergy exceeded thirty days.

Other uniform protocol provisions required that vials of streptomycin or dihydrostreptomycin be equipped by the manufacturer with coded labels. Special report forms for each patient were prepared at the outset, at the 120-day point, and six months after the conclusion of all drug therapy. Audiograms were also recorded at each of these intervals. Accordingly, roentgenographic, laboratory, and clinical evaluation of the patients' status and the progress of the lesions, based on pretreatment findings and follow-up observations, are presented in appropriate sections of the present report.

Finally, it should be noted that para-aminosalicylic acid (PAS) or one of its salts was administered to all patients concomitantly with the streptomycin or dihydrostreptomycin. The daily oral stipulated dosage was to be equivalent to 12 gm. of the acid and was never less than 8 gm.

RESULTS

Clinical Material

Streptomycin or dihydrostreptomycin was administered to a total of 166 patients, but only 141 are included in the clinical appraisal. The reasons for exclusion of 25 patients from the final analysis are presented in a subsequent section of the present report.

Dihydrostreptomycin: A total of 84 patients received dihydrostreptomycin. Eleven of the patients were later excluded from the 120-day clinical analysis for the following reasons: 5 were discharged against medical advice; in 4 the treatment was stopped because of significantly severe drug reactions (to PAS and/or dihydrostreptomycin); a wrong diagnosis was discovered in one; and one patient died.

Of the 73 patients included in the clinical analysis, 3 received dihydrostreptomycin for 120 days only, while the rest received it for more than 120 days.

Streptomycin: A total of 82 patients received streptomycin. Fourteen of the 82 were excluded from the 120-day clinical analysis because of the following factors: 3 were discharged against medical advice; and in 11 the treatment was stopped because of severe drug reactions (to PAS and/or streptomycin).

Of the 68 patients included in the clinical analysis, one received streptomycin for 120 days only and the rest received it for more than 120 days.

At the start of chemotherapy, the distribution of the patients in the two drug groups, according to the extent of pulmonary involvement, was about the same. However, exclusion of cases from the final clinical analysis for the reasons mentioned above resulted in the somewhat dissimilar distribution of cases in the two groups shown in table 1.

Therapeutic Efficacy

Evaluation of the therapeutic efficacy of the two drugs at 120 days (table 2) was based on the usual criteria of change in clinical findings, roentgenographic course, and bacteriologic studies. In addition, the purpose for which chemotherapy was to be used was expressed as the objectives of treatment, with the degree of attainment of these objectives serving as a measure of the value of the drug employed.

To this end, medical staffs established one or more therapeutic objectives for each patient at the outset, and the objectives were entered in the notes of the original conference. The pattern which eventually emerged showed that these objectives almost invariably included one or more of the following goals:

To obtain maximal roentgenographic clearing
To obtain sputum negative for *M. tuberculosis* by culture
To obtain closure of cavity
To effect maximal improvement of a complicating tuberculous lesion
To permit surgery
To provide antimicrobial protection during surgical procedures

TABLE 1

EXTENT OF TUBERCULOSIS AT OUTSET OF CHEMOTHERAPY

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	PULMONARY LESIONS RATED		
		Minimal	Moderately Advanced	Far Advanced
Streptomycin	68	17	45	6
Dihydrostreptomycin	73	30	36	7

TABLE 2

ACHIEVEMENT OF THERAPEUTIC OBJECTIVE

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	DEGREE OF SUCCESS NOTED			
		Complete as Hoped for	Partial but Significant	Slight only	Failure
Streptomycin	68	19	22	22	5
		60 per cent			
Dihydrostreptomycin	73	23	24	22	4
		64 per cent			

The degree of achievement of the objectives of drug therapy, which were graded as complete, partial, slight, or failure, was assessed in staff conference.

Achievement was considered complete in 23 patients (32 per cent) who received dihydrostreptomycin and in 19 (28 per cent) who received streptomycin. Achievement was partial in 24 patients (33 per cent) who received dihydrostreptomycin and in 22 (32 per cent) who received streptomycin. Achievement was judged slight in 30 per cent of the patients who received dihydrostreptomycin and in 32 per cent of those who received streptomycin. Failure was reported in 5 and 7 per cent of the groups, respectively.

At present, surgery or collapse has been performed in 35 patients (48 per cent) who received dihydrostreptomycin and in 26 (38 per cent) who received streptomycin. However, only 3 patients who received streptomycin and 6 who received

dihydrostreptomycin had had surgical measures by the conclusion of the basic 120-day period. Because "to permit the use of definitive surgery" was frequently listed as one of the objectives, table 3 includes a classification of the surgical or collapse measures applied.

TABLE 3

SURGICAL AND/OR COLLAPSE MEASURES EMPLOYED DURING AND AFTER 120 DAYS OF TREATMENT

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY		PROCEDURES					
			Thorocoplasty		Resection with or without Thoracoplasty		Other (Collapse)	
	120 Days or Less	120 Days and More	During 120-Day Period	After 120-Day Period	During 120-Day Period	After 120-Day Period	During 120-Day Period	After 120-Day Period
Streptomycin*	68	67	1	3	2	10	6	4
Dihydrostreptomycin†	73	70	2	6	4	15	3	5

* Earliest surgery: 2 days; latest: 1 year.
† Earliest surgery: 21 days; latest: 1 year.

TABLE 4

ROENTGENOGRAPHIC IMPROVEMENT

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	ROENTGENOGRAPHIC CHANGES OBSERVED		
		Better	No Change	Worse
Streptomycin	68	49 (72 per cent)	15	4
Dihydrostreptomycin	73	58 (79 per cent)	12	3

TABLE 5

SYMPTOMATIC IMPROVEMENT

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY 120 DAYS	SUBJECTIVE CHANGES OBSERVED			
		Better	No Change	Worse	Not Stated
Streptomycin	68	52 (76 per cent)	15	0	1
Dihydrostreptomycin	73	52 (71 per cent)	21	0	0

The roentgenographic abnormalities were classified as improved in 79 per cent of the dihydrostreptomycin group and in 72 per cent of the streptomycin group (table 4).

Symptomatic improvement was noted in 71 per cent of those who received dihydrostreptomycin and in 76 per cent of those who received streptomycin (table 5).

Sputum examinations before drug therapy was started were positive for tubercle bacilli in 93 per cent of those who received dihydrostreptomycin and in 88 per cent of those who received streptomycin (table 6). After 120 days of drug treatment, 27 per cent of the patients who received dihydrostreptomycin were still discharging tubercle bacilli, compared with 16 per cent of the patients who were treated with streptomycin.

These clinical findings reveal no significant difference in the therapeutic efficacy of the two drugs at the end of 120 consecutive days of treatment.

TABLE 6

REVERSAL OF INFECTIOUSNESS

DRUG	NUMBER OF PATIENTS WHO RECEIVED 120 DAYS' THERAPY	SPUTUM FINDINGS								
		Pretreatment				At 120 days				
		Positive for Tubercle Bacilli			Negative	Positive for Tubercle Bacilli			Negative		Not Stated
		Smear	Concentrate	Culture	Culture	Smear	Concentrate	Culture	Concentrate	Culture	
Streptomycin	68	7	17	36	8	2	4	5	25	29	3
		88 per cent				16 per cent					
Dihydrostreptomycin	73	12	24	32	5	1	10	9	9	44	0
		93 per cent				27 per cent					

Drug-Resistant Strains of M. Tuberculosis

Dihydrostreptomycin: Bacterial drug-susceptibility data are available both before and after treatment for the 73 patients who received dihydrostreptomycin for 120 or more days. Except for 5 patients whose sputum contained no tubercle bacilli, the pretreatment organisms were found to be drug susceptible in all but one instance. By drug-susceptible, it is meant that the strain of tubercle bacilli was inhibited by a concentration of 3 γ of dihydrostreptomycin per ml. In one exception, the tubercle bacilli were resistant to 10 γ per ml. At the end of the 120-day treatment period, 20 (27 per cent) of the patients were still discharging tubercle bacilli; of these twenty strains, only four were drug resistant and these were not inhibited by 3 γ per ml. The one strain initially resistant to 10 γ of drug per ml. had become drug susceptible. At the end of this aspect of the investigation, nine months after the last patient was started on drug therapy and twenty-one months after the first patient was so started, 12 patients were still discharging tubercle bacilli. Of these twelve strains, five were resistant to 10 γ of drug per ml., and the remaining seven were completely susceptible to dihydrostreptomycin. Three of the five resistant strains were obtained from patients whose cultures had previously been drug susceptible, both prior to treatment and 120 days thereafter; a fourth strain had been resistant to only 3 γ of drug per ml. at 120 days; and the fifth strain was from a patient whose cultures

were initially resistant to 10 γ per ml. At 120 days, the patient's sputum was negative for tubercle bacilli but subsequently became positive. At the end of the study, the tubercle bacilli from this patient were resistant to 10 γ of drug per ml.

Streptomycin: As for the 68 patients who received streptomycin for 120 days or more, pretreatment bacteriologic study revealed that the sputum of 8 was negative for tubercle bacilli and that the organisms tested from all other sputum specimens were drug susceptible, with two exceptions. The resistant strains were not inhibited by 3 γ per ml. and by 10 γ of drug per ml., respectively. At the end of the 120-day treatment period, 11 (16 per cent) of the patients were still discharging tubercle bacilli, and on 3 others no bacteriologic information was available. Of the 11 patients, 2 had strains which were resistant to 3 γ of drug per ml.; 3 had strains resistant to 10 γ per ml.; and one patient whose strain was initially resistant to 10 γ per ml. became noninfectious. At the close of the study, 8 patients were still discharging tubercle bacilli in the sputum. Resistant strains were present in 3: one whose bacilli were resistant to 10 γ of drug per ml. at 120 days continued to discharge comparably resistant bacilli; a second patient's strain was susceptible at 120 days but was resistant to 10 γ of drug per ml. at the end of the study. The third patient, whose bacilli were resistant to 10 γ of drug per ml. at 120 days, had bacilli which were resistant to more than 100 γ of drug per ml. at the end of the study. The remaining 5 patients yielded tubercle bacilli which remained drug susceptible.

Thus, at 120 days, drug-resistant tubercle bacilli were demonstrated in only 9 of the 31 patients who were still infectious, and in only three strains was the level of resistance as high as 10 γ of drug per ml. Nine months after the last patient was started on drug therapy and twenty-one months after the first patient was so started, only 20 patients, roughly one-seventh of the total, still had sputum positive for tubercle bacilli by any method of examination, and in only one patient were the organisms found to be resistant to 100 γ or more of drug per ml. of medium.

Ototoxicity

Vestibular: In table 7 are shown the frequency, severity, and temporal range of the onset of vestibular disturbances as noted during the initial 120-day treatment period or later. The reactions were rated as slight if vertigo alone was the complaint; moderate, if nondisabling ataxia developed; and severe, when disabling ataxia occurred or the presence of a nonreacting labyrinth was determined.

It is apparent that vestibular disturbances can occur with either streptomycin or dihydrostreptomycin. However, patients treated with streptomycin demonstrated a higher total frequency of such toxic manifestations and, in particular, a much higher frequency of reactions of a more serious nature. Seven of the 84 patients originally placed on dihydrostreptomycin developed slight vestibular abnormalities, compared with 10 of the 82 patients originally assigned to the streptomycin group. Only 2 patients who received dihydrostreptomycin developed reactions, both of moderate severity, but in each instance it was believed

wise to discontinue dihydrostreptomycin. In contrast, 15 patients treated with streptomycin developed reactions of moderate severity, and in 6 of these instances it was deemed advisable to discontinue the drug.

The vestibular disturbances which developed subsequent to the 120-day point seemed to be of comparable incidence and severity. Three patients in the dihydrostreptomycin group and 2 in the streptomycin group were noted to have manifested vestibular disturbances of slight intensity. Three patients who received dihydrostreptomycin and 4 who received streptomycin developed symptoms of moderate severity, for which treatment was stopped in one and three instances, respectively. All of these patients are still under observation, and to date no progression in the severity of symptoms has been noted in any of them.

TABLE 7

INCIDENCE, SEVERITY, AND ONSET OF VESTIBULAR DISTURBANCES

DRUG	NUMBER OF PATIENTS WHO RECEIVED THERAPY		NUMBER OF PATIENTS WITH VESTIBULAR TOXICITY			NUMBER OF PATIENTS DROPPED BECAUSE OF VESTIBULAR TOXICITY
	120 Days or Less	120 Days or More	Slight (Vertigo)	Moderate (Ataxia)	Severe (Dead Labyrinth)	
Streptomycin*	82		10	15	0	6
		67	2	4	0	3
Dihydrostreptomycin† ..	84		7	2	0	2
		70	3	3	0	1

* Earliest discovered: 7 days; latest: 14.5 months.
† Earliest discovered: 15 days; latest: 16 months.

Auditory: For convenience, auditory disturbances were classified in terms of severity, as follows:

> Audiometric change limited to the high tone range and/or sustained tinnitus were scored as *subclinical hearing loss. Clinical deafness* measured according to Fletcher's criterion (average of two smaller values of loss at 500, 1,000 and 2,000 cycles per second) was scored as *slight* if the loss was from 5 to 20 decibels, *moderate* if the loss was from 25 to 40 decibels, and *severe* if the loss was 45 decibels or greater.

Pretreatment clinical and audiometric studies revealed that 7 patients in the dihydrostreptomycin group had a hearing loss before drug therapy was begun. Four of these patients had clinical deafness as well as high-tone hearing loss, whereas the remaining 3 were found to have only an audiometrically detectable hearing loss, confined to high tone ranges. Similar pretreatment study of those in the group to be given streptomycin disclosed clinical deafness in 3 patients and a subclinical, i.e., high-tone, hearing loss in one.

Similar clinical and audiometric studies were made at the completion of 120 days of drug therapy and thereafter (table 8). During the basic treatment period, 9 patients treated with dihydrostreptomycin developed high-tone impairment; in one instance this represented an increase in high-tone hearing loss over that

noted prior to treatment; 2 additional patients complained only of sustained tinnitus, and one other developed slight clinical deafness.

Thus, during the 120-day period, 11 patients who received dihydrostreptomycin incurred subclinical hearing loss, while one additional patient suffered clinical impairment of hearing rated as slight. In contrast, only 2 patients who received streptomycin developed any demonstrable disturbance of hearing and in both the impairment was rated as a subclinical loss. One of the patients was found to have suffered a high-tone hearing loss by audiometric examination, while the other complained only of sustained tinnitus.

Thereafter, audiometric and other studies were repeated at intervals governed by the type and rapidity of onset of auditory damage, but in no instance was this

TABLE 8

INCIDENCE, SEVERITY, AND ONSET OF AUDITORY DISTURBANCES

DRUG	NUMBER OF PATIENTS RECEIVING RX		NUMBER OF PATIENTS WITH AUDITORY TOXICITY					NUMBER OF PATIENTS DROPPED BECAUSE OF AUDITORY TOXICITY
			Subclinical Hearing Loss		Clinical Hearing Loss			
	120 Days or Less	120 Days or More	Audiometric	Tinnitus Only	Slight (5–20 db)	Moderate (25 to 40 db)	Severe (45 db and over)	
Streptomycin*	82		1	1	0	0	0	0
		67	8	3	0	1	0	1
Dihydrostreptomycin†	84		9	2	1	0	0	0
		70	7	1	6	6	0	15

* Earliest discovered: 120 days; latest: 12 months.

† Earliest discovered: 120 days; latest: 16 months.

interval greater than two months. The later findings are also included in table 8. Changes were again evaluated in relation to the time of onset of symptoms, their severity, and the total duration of drug therapy.

Late development of auditory impairment: To date, these later studies have revealed that 20 of the 70 patients who continued to receive dihydrostreptomycin beyond the 120-day point also have developed auditory disturbances for the first time. Of these, 8 had changes rated as subclinical hearing loss, 6 demonstrated slight clinical deafness, and 6 exhibited moderate clinical deafness.

Five other patients, previously reported at the 120-day observation point as having exhibited auditory disturbances, were found in these later studies to have developed changes of even greater magnitude. All of the 5 had only a subclinical hearing loss at 120 days but, when last reported, the disturbance in 3 had progressed to slight clinical deafness, and 2 had developed moderate clinical deafness. (These cases are not detailed in table 8 since such inclusion would constitute a double entry.)

The striking finding was that auditory disturbances were both more frequent and more severe when treatment was continued beyond six months. Eight

patients who received dihydrostreptomycin developed late auditory disturbances which were progressive even after treatment was stopped. In one instance such progression was first noted eight months after cessation of 120 days of drug therapy.

A comparison of the severity of the symptoms and duration of treatment suggests a correlation in so far as symptoms of subclinical hearing loss or mild clinical deafness are concerned. Subclinical hearing loss is common at 120 days when treatment is given for 120 days only, whereas symptoms of clinical deafness rarely appear before the completion of six consecutive months of treatment. Thereafter, clinical deafness ranging from slight to severe is rather frequent. However, the number of cases in this study showing an auditory derangement amounting to moderate or severe clinical hearing loss is entirely too small to permit statistical correlation with duration of therapy. The findings merely suggest that, the longer treatment with dihydrostreptomycin is given, the more likely are the symptoms to be of progressively greater severity.

With respect to streptomycin, 12 of the 67 patients who received that antibiotic for more than 120 days have to date developed late auditory disturbances. Eleven patients were classified as having subclinical hearing loss and deafness, and one developed a moderate deafness. Therapy was discontinued on one patient after eighteen months of treatment with streptomycin because of a slowly progressive loss first noted some months earlier. Three patients with severe clinical deafness prior to treatment showed no measurable change and are therefore not included in the above figures.

Allergic responses: Three patients who received dihydrostreptomycin and PAS developed fever and rash. Seven patients in the dihydrostreptomycin group were found to have eosinophilia of greater than 7 per cent. Ten patients who received streptomycin and PAS developed drug rash and fever, and 8 patients in this group were found to have eosinophilia of greater than 7 per cent. Since subsequent test doses of each of these drugs were not given in every instance, no absolute statement can be made concerning the frequency of allergic responses to streptomycin alone or to dihydrostreptomycin alone.

All of the patients remaining available in both the streptomycin and dihydrostreptomycin groups are being followed with serial audiometric, caloric, and other clinical laboratory studies. The complete results of prolonged treatment and follow-up will be the subject of one or more subsequent papers.

SUMMARY

In a comparative study of streptomycin and dihydrostreptomycin, no difference in the therapeutic efficacy of the two drugs was discernible when they were administered for 120 days and when the results were evaluated at that time.

No significant difference was observed in the frequency of emergence of drug-resistant strains of tubercle bacilli which with either drug was less than 8 per cent. The small percentage of patients in each original group who still had sputum positive for tubercle bacilli after one year of treatment emphasizes the impropriety of drawing any conclusions about the drug resistance studies with respect to the two drugs.

Ototoxicity of the two drugs is of considerable importance:

Vestibular disturbances can occur with either drug but are more frequent, of greater severity, and often of earlier occurrence when streptomycin is employed than when dihydrostreptomycin is used.

Auditory disturbances are relatively uncommon when streptomycin is employed and, when present, are usually of lesser severity.

Auditory disturbances are more to be expected when dihydrostreptomycin is used. They are usually mild or slight in severity when drug therapy is given for only 120 days (or the equivalent). They are of greater severity and frequency when administration is prolonged beyond six months of daily treatment (or the equivalent). Furthermore, the auditory disturbances can progress even after therapy is stopped or they may appear some time after the conclusion of chemotherapy.

The earliest changes of ototoxicity detected with either drug are usually tinnitus and/or high-tone loss of relatively mild degree.

SUMARIO

Estudio Comparado de la Estreptomicina y la Dihidroestreptomicina en la Tuberculosis Pulmonar

No se observó diferencia en la eficacia terapéutica de la dihidroestreptomicina y la estreptomicina administradas durante 120 días, al comparar los resultados al alcanzar ese punto de observación.

Tampoco se notó mayor diferencia en la frecuencia de la formación de farmacorresistencia, que, en ambos casos, fué menor de 8 por ciento. El pequeño porcentaje de enfermos de cada grupo primitivo que tenían todavía esputo positivo al cabo de un año sirve para recalcar lo impropio que es sacar la menor conclusión acerca de dicha resistencia.

La ototoxicidad revistió considerable importancia.

Pueden presentarse trastornos vestibulares con uno u otro antibiótico, pero fueron más frecuentes, más graves y a menudo más tempranos con la estreptomicina que con la dihidroestreptomicina.

Los trastornos auditivos fueron relativamente raros cuando se usó la estreptomicina y, cuando aparecieron, fueron por lo general menos graves.

Los trastornos auditivos fueron más frecuentes cuando se usó la dihidroestreptomicina, pero generalmente de gravedad leve o nula cuando no se administraron más de 120 días (o su equivalente) de farmacoterapia. Mostraron más gravedad y frecuencia cuando se prolongó la administración a más de seis meses de tratamiento diario (o su equivalente). Además, se observó su agravación aun después de suspenderse la terapéutica y, en algunos casos, aparecieron algún tiempo después de terminar aquella.

Las alteraciones más tempranas observadas con ambos antibióticos fueron por lo general zumbidos de oídos y/o pérdida relativamente leve de los tonos altos.

RESUME

Etude comparative de la streptomycine et de la dihydrostreptomycine dans la tuberculose pulmonaire

Aucune différence apparente n'a été constatée dans l'efficacité thérapeutique de la dihydro-streptomycine et de la streptomycine administrées pendant 120 jours quand les résultats étaient comparés au 120ème jour.

Aucune différence significative n'était observée dans la fréquence d'apparition de la résistance à l'antibiotique, qui, dans les deux cas, était inférieure à 8%. Le faible pourcen-

tage de malades, dans chaque groupe original, dont les crachats demeuraient positifs au bout d'une année montre bien combien toute conclusion dérivée des études sur la résistance aux antibiotiques peut être erronée.

L'importance de la toxicité sur l'oreille était considérable.

Les troubles vestibulaires peuvent se produire avec l'un ou l'autre des antibiotiques, mais ils étaient plus fréquents et plus graves et souvent d'apparition plus précoce avec la streptomycine que lorsque la dihydrostreptomycine était administrée.

Les troubles de l'audition étaient relativement rares quand la streptomycine était utilisée et, quand ils se produisaient, leur sévérité était habituellement moindre.

Les troubles de l'audition étaient plus fréquents quand la dihydrostreptomycine était utilisée habituellement d'une sévérité modérée ou minime quand l'antibiotique n'était administré que pendant 120 jours (ou leur équivalent). Leur sévérité et leur fréquence étaient plus grandes quand l'administration quotidienne était prolongée au-delà de six mois (ou leur équivalent). De plus, on a observé leur progression après la cessation du traitement; dans certains cas, ils se sont produits un certain temps après la fin de la chimiothérapie.

Les altérations les plus précoces qui aient été observées avec l'un ou l'autre de ces antibiotiques étaient habituellement les bourdonnements d'oreilles, la perte des sons élevés à un degré relativement modéré ou l'une et l'autre de ces réactions.

Acknowledgment

Grateful acknowledgment is made to the many who assisted in the study and in the completion and analysis of the data. In particular we should like to express our appreciation to Dr. Charles E. Lyght, Dr. Joseph E. Hawkins, Jr., and Miss Betty M. Repassy of Merck & Co., Inc.; to Dr. H. McLeod Riggins of New York City; and to Mr. Ben Locke, statistician, and the secretarial staff of the Division of Tuberculosis, New York State Department of Health.

Case Reports

DRUG FEVER DUE TO ADMINISTRATION OF ISONIAZID[1]

ALEXANDER KRASNITZ

(Received January 26, 1953)

INTRODUCTION

Numerous toxic reactions have been reported by the early investigators in connection with the administration of the hydrazine derivatives of isonicotinic acid (1, 2, 4–7, 9). In referring to toxic reactions in general, a distinction must be made between isoniazid, known under various trade names, and iproniazid, the isopropyl derivative of isonicotinic acid hydrazide. With iproniazid, drug toxicity has been encountered more often and more intensely than with the mother substance, isoniazid (5). A careful search of the literature, however, fails to reveal any febrile reaction in connection with the therapeutic use of isoniazid in this country. To be sure, Tanner and his associates (8) in Switzerland mentioned the occurrence of drug fever in one of 100 cases of pulmonary tuberculosis and in fourteen cases of extrapulmonary tuberculosis treated with isoniazid. A febrile reaction in the course of administration of isoniazid apparently is a rare occurrence. A related chemical derivative of nicotinic acid, pyrazinamide,[2] used in the treatment of pulmonary tuberculosis in 43 cases by Yeager, Munroe, and Dessau (3), was reported to have given febrile reactions in three patients. It is not within the scope of this presentation to account for the mechanism of such febrile reactions. It is believed, however, that they represent allergic reactions.

The following case report includes a series of events which are believed to represent febrile reactions to isoniazid.

CASE REPORT

M. C., a 62-year-old white female, was admitted to the tuberculosis hospital on April 4, 1952, with a diagnosis of moderately advanced, active, pulmonary tuberculosis of five years' duration. A roentgenogram of the chest showed a fibro-ulcerative lesion in the right upper lobe. Prior to admission to the hospital, the patient had been treated at home with streptomycin and bed rest. She discharged *M. tuberculosis* intermittently in the sputum. The most recently examined sputum specimens, however, were positive for acid-fast bacilli, and strains resistant to streptomycin were isolated. On admission to the hospital, the patient appeared somewhat undernourished and chronically ill. Her height was 62 inches and she weighed 100¼ pounds.

On April 7, 1952, the patient was started on isoniazid, 100 mg. twice daily at twelve-hour intervals. Prior to initiation of this therapy, the patient was afebrile, with only an occasional rise of temperature to 99°F. The cephalin-cholesterol flocculation reaction (forty-eight hours) was negative. The nonprotein nitrogen was 40.5 mg. per 100 ml. of blood. Urine analysis revealed no abnormalities. Blood count: erythrocytes, 4.65; leukocytes, 9.29 per cu. mm.; hemoglobin, "89 per cent." Differential count: polymorphonuclears, 57;

[1] From Estelle and Walter C. Odell Memorial Sanatorium, Orange County Tuberculosis Hospital, Newburgh, New York.

[2] Used in this instance as Aldinamide, the proprietary name of American Cyanamid Company.

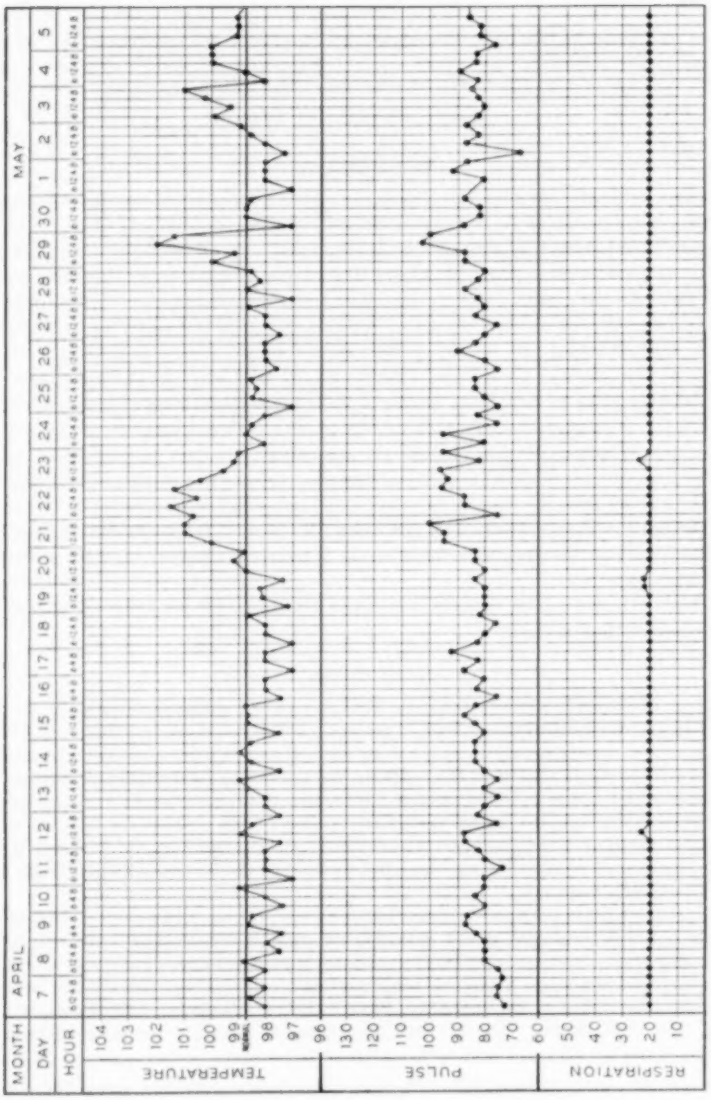

FIG. 1. Temperature chart of patient M. C. (No. 2454), 1952

lymphocytes, 37; monocytes, 2; eosinophils, 2; basophiles, 1; hematocrit, 41 per cent. The erythrocyte sedimentation rate (Wintrobe) was 12 mm. per hour. On the day isoniazid therapy was initiated, the patient's temperature was 98°F. and the pulse and respiration were 72 and 20 per minute., respectively.

On April 21, 1952, after fourteen days of therapy with isoniazid, the patient developed a temperature of 101°F., with a pulse of 100 and respiration of 20, and complained of malaise and pain in the legs and back. There were no symptoms or complaints referrable to the chest. During the period of therapy with isoniazid, no other drugs were administered save for milk of magnesia and an oil enema, given for constipation. Because of the rise in temperature, the isoniazid therapy was discontinued on April 22, 1952. Three doses of acetylsalicylic acid and acetophenetedin compound tablets were then given. The temperature fell by lysis and, within two days, was 98.8°F.

On April 26, 1952, isoniazid therapy was resumed; the same dosage of 200 mg. daily was given. On April 29, 1952, three days later, the patient's temperature again rose to 102°F., the pulse to 105 per minute, while the respiration remained unchanged. The patient complained of headache and chills. As it was believed that the isoniazid therapy was probably the contributing factor, it was again discontinued temporarily on the same day. On the following morning, the temperature fell to 97°F. and remained normal or 99°F., as was usual for this patient. A urine analysis during the febrile period showed a few pus cells, but was otherwise not abnormal. No therapy for the fever had been administered because of this episode.

On May 2, 1952, isoniazid therapy was resumed with the same dosage as was previously used. The temperature rose to 100°F. and the following day to 101°F. The pulse remained 80 to 90 and the respirations at 20 per minute. The medication was continued, however, and the temperature stayed at 100°F. for four days. On the following day it fell to 99°F. and remained normal or 99°F. for the remainder of the therapy. Isoniazid therapy was continued until October 16, 1952, or for a period of six months. It must be added that examination of the patient during each of the febrile episodes mentioned above failed to disclose any evidence of spread of the tuberculous lesion or any intercurrent infection which might have produced the fever.

Repeated nonprotein nitrogen determinations, urine analyses, and cephalin flocculation tests failed to show any abnormalities. Nonprotein nitrogen ranged between 23 and 40 mg. per 100 ml. of blood, and the cephalin-flocculation reaction (48 hours) remained negative. The patient developed a mild hypochromic anemia, with erythrocytes, 3.61; leukocytes, 7.30 per cu. mm.; hemoglobin, "73 per cent," and a differential count of polymorphonuclears, 67; lymphocytes, 30; eosinophils, 3. The hematocrit was 42 per cent.

At the time the patient received isoniazid there were 3 other patients receiving this therapy. Very shortly thereafter, another group of 5 patients received the drug. The dosage regimen consisted of 4 to 5 mg. of isoniazid per kg. of body weight; no febrile reaction was noted in any of the patients except the subject of the present report. As the rise in temperature appeared fourteen days after therapy, it was believed that the patient was sensitized to this drug during this period and that the febrile reaction was, therefore, on an allergic basis. Although the reaction necessitated interruption of the treatment on two occasions, it was not considered serious and, to be sure, did not require any further interruption of therapy after the third febrile episode. The ability to continue the administration of isoniazid despite sensitivity to it is not an unusual occurrence, and it is well recognized that a certain percentage of patients who show sensitivity to drugs, especially penicillin, are subsequently able to take the same drug without again showing such sensitivity phenomena.

SUMMARY

The case of a patient who developed febrile reactions during the course of isoniazid therapy is reported. While the reactions occurred on three occasions, it was possible, nevertheless, to continue this drug without further interruption and without untoward effect.

Such febrile reactions to isoniazid are believed to be rare and presumably represent a reaction of the allergic type.

SUMARIO

Fiebre Medicamentosa Debida a la Administración de la Hidracida del Acido Isonicotínico

Descríbese un caso de reacción febril en el transcurso de la isoniazidoterapia. Aunque recurrió aquélla en tres ocasiones, fué posible continuar la medicación sin más interrupción y sin efecto adverso. Según parece, esta ocurrencia es rara y de la naturaleza de una reacción alérgica.

RESUME

Fièvre médicamenteuse à la suite de l'administration d'hydrazide de l'acide isonicotinique

Un cas de réaction fébrile au cours de la thérapie par l'isoniazide est rapporté. Malgré la récurrence de ce phénomène à trois reprises différentes, l'administration de cette préparation a pu être poursuivie sans autre interruption et sans réaction nocive. Cet accident est considéré comme rare et de nature allergique.

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ACUTE HEMATOGENOUS TUBERCULOSIS SECONDARY TO PREGNANCY IN A PATIENT WITH TUBERCULOUS SALPINGITIS[1]

Case Report and Review

HOWARD G. TURNER, JR.

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INTRODUCTION

The interrelationship of pulmonary tuberculosis and pregnancy has been the subject of considerable discussion in the literature on tuberculosis. In contrast, comparatively little information has been published concerning the relationship of tuberculous disease in the female genital tract and pregnancy. Perhaps because of the well-ingrained axiom that tuberculous salpingitis and sterility are synonymous, the occurrence of pregnancy in the presence of pelvic tuberculosis has been considered a clinical rarity, if not an impossibility. Nevertheless, review of the available pertinent literature reveals that such cases do occur and that there are inconsistencies in the evaluation of the pathogenesis of this clinical situation.

Although there seems to be no proved causal relationship between the physiologic alterations of pregnancy and possible changes in host response to infection with the tubercle bacillus (1) such as occur in the diabetic (2, 3), there can be a directly related and often fatal series of events when pregnancy occurs in a woman with a significant focus of tuberculosis localized in the genital tract. The following case is illustrative of the situation and its complications, and is presented with a review of the literature in an effort to clarify and emphasize the problem.

CASE REPORT

History:. The patient was a 37-year-old Negro woman who was admitted to the hospital on October 30, 1950, because of a diagnosis by roentgenogram of miliary tuberculosis. She had had a spontaneous delivery of a six and one-half months' old baby, weighing three and one-half pounds, five weeks previously. The pregnancy had been uncomplicated, as was the delivery. However, the child developed a cough and died of generalized tuberculosis at the age of five weeks, the diagnosis being confirmed by pathologic findings. Unfortunately, the placenta was not examined. The mother was asymptomatic so that the hospital staff personnel were suspected as the source of infection, and roentgenograms were obtained of them as the possible source of infection before the miliary pattern was seen in the mother's film. In retrospect, the only illness remembered by the patient occurred approximately one month before her pregnancy began and was characterized as an upper respiratory infection with fever which lasted about a week. Other than this, there had been no known illness or febrile period, including the time of delivery and up until the time of her admission to the sanatorium. She had had no known recent exposure to tuberculosis. A roentgenogram of the chest in 1949 was reported to have been normal. There was no evidence of tuberculosis in the husband. The patient's menstrual history indicated periods at regular intervals without unusual symptoms or flow, with onset at the age of 15. It is to be noted, however, that over the twelve-year period of her marriage no pregnancy occurred although there had been no effort to prevent this. Minor investigation had been made to discover reasons for the failure, but this did not include a curettage. The childhood illnesses included measles and whooping cough, but without known sequelae, and she had had no known childhood tuberculous infection.

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[1] From Uncas-on-Thames, State Tuberculosis Sanatorium, Norwich, Connecticut.

Physical examination: Physical examination showed an apparently well Negro woman. The temperature was normal. Examination of the eyes was normal, and no tubercles were seen on careful search of the fundi. There were no palpable lymph nodes. The chest cage moved well, percussion note and breath sounds were normal, and there were no audible rales in either lung. The abdomen was flat, soft, and nontender; neither spleen, liver, nor kidneys was palpable. The pelvic examination showed minimal milky white vaginal discharge. No adnexal masses could be palpated. There was no tenderness on motion of the cervix and the uterus appeared to be normal in size. Neurologic examination revealed no abnormalities of the cranial nerves, no nuchal rigidity, equal and normal reflexes throughout, and no sensory abnormalities.

The cutaneous reaction to tuberculin (first-strength PPD) was positive. The urine showed a few leukocytes on microscopic examination (catheterized specimen). Cultures of the urine for various bacteria and three 24-hour specimens cultured for tubercle bacilli were sterile. The vaginal discharge and menstrual blood specimens were smeared and cultured for tubercle bacilli, and none was found. A series of nine 72-hour sputum collections were concentrated and cultured for *Mycobacterium tuberculosis*. All of these were negative. Blood counts were unremarkable. The roentgenogram of the chest showed a typical miliary pattern of hematogenous tuberculous infiltration.

Clinical course: On November 2, 1950, the patient started to receive 1 gm. of streptomycin intramuscularly and 12 gm. of para-aminosalicylic acid (PAS) by mouth daily. The cerebrospinal fluid before treatment showed normal pressures, no cells, and 67.5 mg. of sugar per 100 ml. The Pandy reaction was negative. Cultures of the cerebrospinal fluid yielded no tubercle bacilli. During the three-month period following the initiation of treatment, the roentgenographic abnormalities receded to such a degree that the films appeared almost normal. During the third month of chemotherapy, however, the patient began to complain of headache and had a temperature elevation. A lumbar puncture on January 19, 1951, showed an initial pressure of 250 mm. of water. There were 45 cells per cu. mm., all leukocytes, and the sugar concentration was 38.5 mg. per 100 ml. A culture of this specimen of cerebrospinal fluid was reported positive for tubercle bacilli on March 15, 1951. On January 24, 1951, the patient's streptomycin dosage was increased to 2 gm. intramuscularly daily and she was started on a course of intrathecal streptomycin in 100 mg. doses given three times weekly. In all, to the time of her death, she received 126 gm. of streptomycin intramuscularly and 0.9 gm. of streptomycin intrathecally.

Throughout this time the only signs of meningitis were headache and temperature elevation. On January 29, 1951, she was noted to have slurred speech and a right-sided hemiplegia. She became comatose, had a series of convulsions in the left leg and arm, became incontinent of urine and feces, and died on February 15, 1951, three and one-half months after admission. The cerebrospinal fluid sugar concentration ranged between 30 and 40 mg. per 100 ml. and the pressure steadily became higher throughout this period. Two of six specimens of cerebrospinal fluid cultured at approximately weekly intervals yielded tubercle bacilli.

Post-mortem examination: A complete autopsy was performed by Dr. Robert Tenant.[1] The gross examination revealed normal-appearing lungs externally. On cut section, however, nodules were scattered uniformly throughout all lobes. There was a well-encapsulated fibrocaseous mass present, one centimeter in diameter, which was in the right lower lobe (figures 1*A* and 1*B*). One lymph node in the right hilar area showed evidence of old fibrocalcific tuberculosis (figure 1*C*). There was no evidence grossly of tuberculous infection in the spleen, liver, adrenals, or kidneys. The peritoneum was studded with 2 mm. nodules.

The pelvic organs were of interest. A small fibroid tumor was present in the fundus, the ovaries appeared normal except for tubercle-like nodules covering the serosal surface. The fimbriated ends of both tubes were free, the right tube was slightly thicker than the

[1] Hartford Hospital Pathology Department.

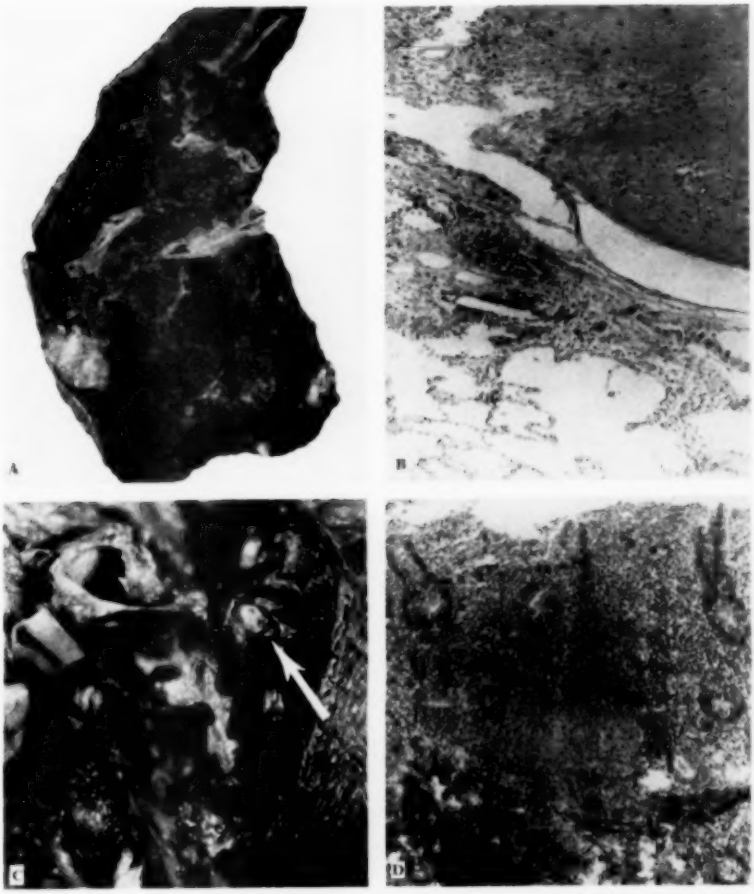

FIG. 1. *A*. Encapsulated primary tuberculous focus in the right lower lobe.

B. Microscopic section through the edge of the lesion seen in figure 1*A*, showing the fibrous capsule, normal surrounding lung parenchyma, and an absence of inflammatory cells.

C. Magnified view of part of the right hilar dissection. The arrow indicates the single fibrocalcific tuberculous lymph node found at autopsy.

D. Section through the endometrium showing tuberculous infiltration with a caseous focus in the deeper layers and indicating the probable source of amniotic fluid infection.

left, but neither was dilated. In cross section, tuberculosis could not be identified grossly. The uterus was not remarkable. One thickened 0.5 cm. area was noted in the fundus.

The brain appeared swollen and had flattened convolutions. Exudate was present at the base around the sella turcica and the peduncles. This was thought to be typical of

tuberculous meningitis. Tubercle bacilli were cultured directly from the surface of the meninges.

The microscopic findings verified the impression of the macroscopic examination. In the lungs tubercle formation was noted in areas approximately one millimeter in diameter

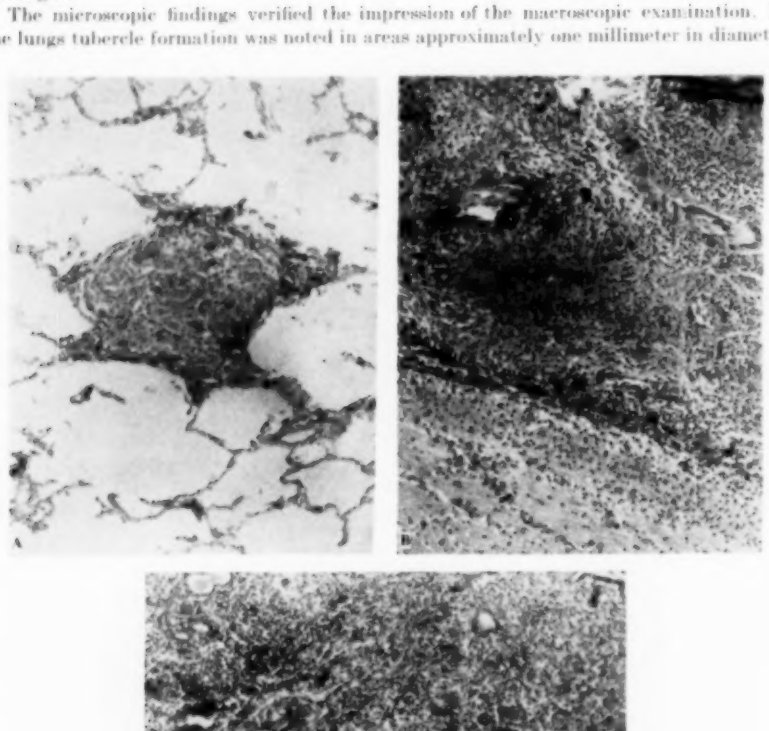

FIG. 2. *A*. A single miliary lesion in the lung after three months of streptomycin and para aminosalicylic acid (PAS) treatment.

B. Cerebral lesion showing arteritis and adjacent tuberculous focus.

C. Section through the ovarian tube showing extensive tuberculous salpingitis.

with caseous centers typical of hematogenous dissemination (figure 2*A*). Section through the area of the caseous mass in the right lower lobe showed a lesion encapsulated by fibrous tissue which contained some calcification, with no evidence of recent local bronchogenic spread. The genital organs showed a marked involvement. There was an extensive endosalpingitis with involvement of the entire mucosa of the tubes (figure 2*C*). Caseous foci were present. The tubal lumina were markedly narrowed. The serosal surface of all of the organs

was involved in the process. The ovaries were not involved, and the myometrium of the uterus was normal. The endometrium contained foci of tuberculosis in the deeper layers. These appeared to be of an acute variety with early necrosis (figure 1*D*).

Sections of the brain tissue showed an extensive tuberculous arteritis, which involved all layers of the vessels, and adjacent parenchymal lesions (figure 2*B*). The meningeal involvement extended to the spinal cord and included both intradural and extradural foci at the point of exit of the nerve roots. In the cerebellum a well-defined area of infarction was noted, with almost complete obliteration of the vessel leading to the area.

Widely scattered foci of hematogenous origin were seen in the spleen, liver, and kidneys. The final diagnoses were:

fibrocalcific primary tuberculosis of the lung
tuberculous salpingitis
tuberculous endometritis
acute hematogenous tuberculosis with involvement of lungs, meninges, and peritoneum

Post-mortem examination of the 5-week-old infant: Autopsy[3] was also performed on the body of the child. Miliary tubercles were found in the liver and spleen, but none in the other organs or in the meninges of the brain. The lungs contained confluent caseous foci throughout. The mediastinal lymph nodes were almost universally involved in a caseous process. The final diagnoses were:

caseous tuberculous bronchopneumonia, secondary to aspiration
miliary tubercles in liver and spleen

DISCUSSION

The true incidence of disseminated tuberculosis today is unknown, but any discussion of tuberculosis of the pelvic organs must of necessity involve this question since hematogenous dissemination at some time is presumed to account for practically all tubal foci (4). In an average sanatorium practice today the number of cases of post-dissemination hematogenous tuberculosis with chronically active foci is apparently diminishing. In 1947, however, Spain (5) reported that 34 per cent of 603 autopsied patients who had died from tuberculosis showed evidence of disseminated lesions, a not inconsiderable number. In addition, tuberculosis in the male genital tract is considered to be more common than in the female (6), but this difference may be more apparent than real considering that the disease is so much more readily discoverable in the male. Regardless of a declining incidence or any sex difference, it must be assumed that hematogenous disseminated foci are present in an unknown but probably appreciable number of women with positive tuberculin reactions, and a certain number of these foci will be in the pelvic adnexa.

According to Novak (7), 85 to 90 per cent of tuberculosis of the female genital organs are tubal in origin and this amounts to approximately 5 to 7 per cent of all cases of salpingitis. He also states that bilateral infection and sterility are the rule. In the recorded series in the literature, the per cent of bilateral infection reported varies from 72 to 98.9 per cent. The variation in these figures probably depends on the thoroughness of the microscopic search for active foci of disease in the tubes, and the higher figure, which indicates almost universal bilaterality of infection, is more likely the accurate one. Hence, unilateral disease which leaves a normal contralateral tube as an impregnation route is probably unimportant. As for the occurrence of pregnancy in this group with pelvic disease, only tubal gestation is mentioned. In statistical studies from the Johns Hopkins Hospital of 402 cases of tuberculous salpingitis and 516 tubal pregnancies, it is reported that only one of these abnormal implantations had tuberculous salpingitis as the basic

[3] Information is supplied through the courtesy of Dr. Averill Liebow, Yale University Medical School. Autopsy was performed at the Lawrence and Memorial Hospital, New London, Conn.

defect (8, 9). These figures are derived from 46,700 pathologic specimens, the whole of the Hopkins pathology collection at that time. At Jefferson Medical College, Bland (10) found another case of tubal gestation secondary to tuberculous salpingitis, the only such case in a review of 55,000 surgical specimens which included 209 tubal pregnancies.

Actually only ten tubal gestations caused by tuberculosis had been recorded in the literature through 1940 and there has been only one more reported since in the American literature (8), a total of eleven documented cases.

Stevenson and Wharton (9), in commenting on the generally accepted theory for the occurrence of the tubal implantation, assume enough patency of the tubal lumen to allow for fertilization. Nevertheless, they visualize the presence of blind pouches and alleys where scarring, regeneration of epithelial lining with fusion of layers of the mucosa, and sometimes constriction from tuberculous infection of the overlying peritoneum have distorted the tubal lumen, have prevented successful passage of the ovum through the tube, and have created a lack of sufficient normal mucosa even to allow for tubal implantation.

No mention is made in the above reviews of the possibility of uterine gestation, and apparently it is assumed that this is even more rare than tubal implantation in the presence of salpingitis. In support of this idea, it is stated in Williams' textbook (11) that, in a review of several thousand routine studies of placentas, none was tuberculous. In 1939 Schaeffer (12) reported from the Sea View Hospital in New York a single tuberculous placenta from a series of 150 tuberculous pregnant women. The woman had far advanced pulmonary tuberculosis and this was assumed to be the disseminating source for a 7 by 7 mm. caseous focus found in the placenta. The only information about the pelvic organs is that "the pelvic examination was negative" six months after the delivery, which was normal. The child was normal and was a negative reactor to tuberculin. Schaeffer believed this to be the only recorded case of identified tuberculous tissue in the placenta since 1922, when Whitman and Green (13) were able to find a total of forty-six cases including two of their own. Although the discussion of Whitman and Green was concerned with other phases of the problem, their figures serve to record the number of cases in which bacteriologic and histologic tuberculosis have been identified in the placenta. Unfortunately they do not attempt to specify the site of primary or major infection in the mother, nor do they mention the postpartum fate of either the mother or child, and this information is also not available in one of their major source references (14), so that the portion of infections arising from pulmonary as against genital foci is undetermined in their cases.

It is well recognized that many patients with pulmonary tuberculosis complete normal uncomplicated pregnancies with proper pre- and post-partum care. Although in 1930 Matthews and Bryant (15) reported that toxic or chronically debilitated tuberculous pregnant women had a higher incidence of abortion, premature birth, and postpartum hemorrhage than nontuberculous women, chemotherapy has reduced the conditions which predispose to these complications to a negligible point today. In contrast, in the reported cases of genital tuberculosis associated with pregnancy, including a few in the era of modern therapy, the end result has been almost universally unfavorable. Nevertheless, there are a few recorded cases in which either the mother or child or both have escaped or recovered from clinical tuberculosis (12, 16). However, Greenberg, in a complete analysis of 200 cases of surgically treated tuberculous salpingitis, includes statistics concerning this disease and pregnancy (17) which are of the greatest interest. Only 61 per cent of the women were sterile; of the others, there was a total of 170 pregnancies in 58 women. One had eleven normal pregnancies. There were no ectopic pregnancies in the group, and no postpartum complications were reported by the patients. There was, of

course, no proof that the tuberculous tubes, removed at a later date, were present during all of these pregnancies, but it seems likely that some of the women at least were harboring foci of tuberculosis acquired prior to the child-bearing age. These figures suggest that normal pregnancy can occur in the presence of tuberculous pelvic disease. Unfortunately, Greenberg did not analyze this material in the light of determining the amount of tuberculosis present in the tubes of these patients at the time of their removal. He does make the general comment, however, that "inasmuch as only a small section of one or the other tube may be involved, many sections must be cut" in order to identify the tuberculous process in some cases. The degree of infection, then, and the location may be important factors in determining both the ability to become pregnant and the site of the impregnation.

It should also be noted that some investigators have suggested that tuberculous endometritis occurs in isolated form in 10 per cent of the cases of pelvic tuberculosis (18, 19). Salpingitis was not carefully searched for in these cases, however, and it seems likely that the tubes were the disseminating focus for the uterine disease, again making the salpingitis the primary defect and the determining factor as far as pregnancy is concerned. When there is tubal disease of an extent sufficient to cause symptoms, it seems certain that pregnancy seldom occurs or, if it does, it ends in disaster (20). Asymptomatic bilateral salpingitis of minimal extent may form a larger group than has been suspected, one in which there is no interference with the normal intrauterine impregnation sequence and one in which the foci in the tubes may remain undisturbed by the physiologic alterations of pregnancy. In this way a successful termination of pregnancy might be obtained without activation of, or complications from, the potentially dangerous foci in the tubes. There is at least indirect evidence that this occurs more frequently than has been commonly believed (17). Conversely, it may be that all hematogenous disseminations of tuberculosis which occur during pregnancy arise from hidden tubal disease even though obvious other extragenital foci may be present. This is both speculative and difficult of absolute proof.

With respect to the complications of tuberculosis in pregnancy, spread of infection is the basic issue, either to the fetus or in the mother. The child may become infected either via the blood stream, by aspiration, or presumably by both routes, as in the present case. Miliary tuberculosis as a complication of pregnancy was studied by Lundstrom (20), who was able to find a total of seventy-two reported cases through 1944. His analysis indicated an extragenital origin of the miliary dissemination (mainly pulmonary) in forty-six. The origin was probably genital in four and definitely so in twenty-two; proof again that pregnancy does occur in the presence of pelvic disease. All of the patients died. In those cases which were examined post mortem, it was noted that, whenever the endometrium was involved, the tubes were always bilaterally involved. It should also be noted that it is not known how carefully the tubes of the patients classified as having pulmonary dissemination were examined for active foci of tuberculosis, and many of these could have had hidden tubal foci which were not detected and which could have acted as the disseminating source.

Until the advent of chemotherapy, therefore, miliary dissemination in this group was universally fatal when it occurred, and this prognosis corresponded to that of miliary disease in general. In the single case published to date with postpartum dissemination treated in the modern era of therapy, the mother died in spite of streptomycin therapy. Although this is an isolated instance (16), there was a similar failure of treatment in the present case in spite of early and energetic measures. Presumably the prognosis in this special group will improve as the treatment of acute disseminated disease improves.

In regard to treatment of tuberculous pelvic disease prior to pregnancy, complete surgical removal of the tubes and uterus has been advocated as the treatment of choice when there is clinically evident tuberculous salpingitis (21, 22, 23; not a complete listing). Repeated curettage has been reported to have accomplished a cure of endometrial infection and to have been followed by uncomplicated pregnancy (18). This appears to be "blind" therapy in that possible persistent tubal foci are ignored, and is a dangerous therapy in that hematogenous dissemination is known to follow the procedure in some cases. Radiation has been reported to have effected cures and allowed for pregnancy, but also to have caused obstructed labor due to fibrosis (19). It would be anticipated that X-ray sterility would result in this situation as a rule. Estimate of the value of antimicrobial agents is not well known as yet (24). In the treatable or, in other words, detectable symptomatic salpingitis cases, however, a chronic caseous state of significantly large foci of tuberculosis has presumably been reached. Hence, chemotherapy might not produce the relatively normal and patent tubal passage required for pregnancy or suppress the disease permanently.

As a consequence, the surgical approach in conjunction with chemotherapy seems the treatment of choice. Few or no pregnancies would be prevented by salpingo-hysterectomy when the pelvic disease is sufficiently advanced to cause clinically evident symptoms. Moreover, the almost universally fatal result of the rare pregnancy which does occur justifies the surgical procedure in any event. In lesser degrees of disease, chemotherapy may offer sufficient protection to justify allowing a pregnancy to occur, and the development of hystero-salpingography (4) and the more widespread use of culture of diagnostic curettings (18) should bring more such cases to light. In these cases, if the acquisition of more information confirms the early belief that antituberculous drugs given ante partum are not harmful to the child, chemotherapy in some form should probably be administered throughout the pregnancy and in the postpartum period.

SUMMARY

The course of a patient with acute hematogenous dissemination of tuberculosis which arose as a consequence of the effects of pregnancy on latent tuberculous salpingitis is described with the autopsy findings. In reconstructing the pathogenesis of the case, a healed primary focus in the lung represented the original infection and the source for the establishment of the endosalpingitis. The pelvic disease was apparently reactivated by the pregnancy to cause endometritis, fetal tuberculosis, and acute postpartum dissemination in the mother. The case represents a failure in treatment despite early and energetic use of the antituberculous drugs available at the time.

It is reasonable to assume that symptomatic tuberculous pelvic disease prevents pregnancy or produces serious complications when it occurs, as in the present case. Nevertheless, there is evidence to suggest also that pregnancy can and does occur in the presence of latent tuberculous salpingitis without disrupting potentially dangerous foci, although this fact has not been emphasized in the literature.

Tuberculosis of female genital organs originates as a hematogenous dissemination to the tubes in the great majority of cases. Although the presence of tuberculous salpingitis may lead to sterility, the location, quantity, and state of activity of the tuberculous disease determine the functional capacities of the reproductive system and the occurrence of late complications.

Treatment of genital tuberculosis should consist of surgical extirpation of the pelvic organs in the symptomatic group of salpingitides. If lesser degrees of infection are dis-

coverable, chemotherapy may be sufficient to control the disease and allow for normal pregnancy and successful delivery.

SUMARIO

Tuberculosis y Embarazo. Presentación de un Caso y Discusión de las Complicaciones de la Tuberculosis Pelviana

A los casos previamente comunicados, agrégase ahora otro caso de diseminación hematógena aguda de la tuberculosis, secundaria a los efectos del embarazo sobre una salpingitis y una endometritis tuberculosas.

Después de un repaso de la literatura, discútese la patogenia de esta situación algo extraña.

Ofrécense sugestiones relativas al mejor modo preventivo y terapéutico de abordar la situación.

RESUME

Tuberculose et gestation. Rapport sur un cas et discussion sur les complications de la tuberculose pelvienne

Un cas additionnel de dissemination hématogène de la tuberculose, due à l'action de la grossesse sur une salpingite tuberculeuse avec endométrite, vient s'ajouter aux cas précédemment cités.

L'article comporte un compte-rendu de la littérature et une étude de la pathogénèse de cette situation relativement exceptionnelle.

Des mesures appropriées et la manière d'envisager la thérapeutique sont suggérees.

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Editorial

Cooperative Clinical Research in Tuberculosis

Medical history has recorded no endeavors comparable to those which have been directed toward the solution of problems arising from the clinical use of streptomycin. The most extensive series of projects have been those led by physicians in the United States Veterans Administration, the most precise studies have been those reported by the British Medical Research Council, and among the pioneer cooperative study groups were those coordinated by the United States Public Health Service and the American Trudeau Society.

This issue of THE AMERICAN REVIEW OF TUBERCULOSIS reports a new series of studies with some unique methods of approach. In comparing streptomycin with dihydrostreptomycin, the identity of the drugs used was masked, thus obtaining a degree of freedom from influence of prior opinion. In addition, the therapeutic goal was defined for each patient prior to treatment, and this record of prediction was filed in a central office at that time. Subsequent comparison of predicted accomplishments with actual performance supplied more objective data than would be supplied by conventional methods. The remarkably similar scores reported for the two drugs in question may be pure coincidence but suggest that the method is a sound one. These data appear adequate to settle the smouldering controversy over the relative efficacy of the two drugs in question which have yielded identical data in laboratory studies, both *in vitro* and *in vivo*. Thus, confidence in laboratory data which bear on clinical studies is again restored.

Cooperative clinical research as applied to problems of tuberculosis therapy have been so eminently successful, regardless of the sponsoring agency, that other fields of clinical research should take more cognizance of this as a means to advance knowledge. While similar end results would eventually appear from more conventional studies, the time required to ascertain the truth would be greatly prolonged.

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Notes

CONTROL STUDY OF COMPARATIVE EFFICACY OF ISONIAZID, STREPTOMYCIN-ISONIAZID, AND STREPTOMYCIN—PARA-AMINOSALICYLIC ACID IN PULMONARY TUBERCULOSIS THERAPY

IV. Report on Forty-Week Observations on 583 Patients with Streptomycin-Susceptible Infections

In the Public Health Service control study of isoniazid[1] among 1,500 patients with pulmonary tuberculosis, those patients who entered the study earliest have completed forty weeks of the prescribed drug therapy. Preliminary results are reported here for 583 of these patients who had streptomycin-susceptible[2] infections at the beginning of the trials.

The plan of the study in which physicians[3] in twenty-two hospitals in the United States have participated has been outlined in previous Notes. Three drug regimens, streptomycin with para-aminosalicylic acid (PAS), streptomycin with isoniazid, and isoniazid have been compared. Streptomycin has been given in dosages of 1 gm. twice a week; PAS, 10 to 12 gm. daily; and isoniazid, 150 to 300 mg. daily. The protocol provides for a sixty-four-week period of observation; a forty-week control period, during which only the chemotherapy determined by chance is used; and a twenty-four-week post-treatment observation period.

A total of 791 patients with streptomycin-susceptible infections came under study early enough to have completed forty weeks of treatment. However, 208 of these patients have been excluded from the present analysis because they did not, for one reason or another, complete the prescribed term of treatment. In the following table, the specific reasons for their exclusion are presented.

REASON FOR EXCLUSION	REGIMEN			
	Total	Streptomycin-PAS	Streptomycin-Isoniazid	Isoniazid
All reasons	208	82	62	64
Left hospital	153	61	45	47
Change in therapy				
Refused	7	5	1	1
Drug intolerance	27	11	10	6
Critical worsening	9	4	—	5
Error	8	1	2	5
Nontuberculous deaths	4	—	4	—

Among the remaining 583 patients, 183 received streptomycin plus PAS; 195, streptomycin plus isoniazid; and 205, only isoniazid. At the beginning of treatment, the char-

[1] The isoniazid for this investigation was generously supplied by E. R. Squibb & Sons as Nydrazid.

[2] Determinations were made by the indirect culture method by Mr. William Steenken, Jr., in the Trudeau Laboratory. Cultures were considered susceptible to streptomycin when growth in the 10 γ per ml. concentration of streptomycin was less than the three- or four-plus growth in the control at five weeks.

[3] The investigators were enumerated in the April, 1953, issue of the REVIEW, page 539.

acteristics of the patients and their disease were similar in the three treatment groups. Nearly two-thirds (63 per cent) had advanced cavitary disease; one-third (33 per cent),

TABLE 1

PERCENTAGE DISTRIBUTION OF ROENTGENOGRAPHIC CHANGE IN 583 PATIENTS WITH STREPTOMYCIN-SUSCEPTIBLE INFECTIONS

	CHANGE FROM BEGINNING OF TREATMENT								
	Total	Improvement			No Change	Deterioration			Death*
		Marked	Moderate	Slight		Slight	Moderate	Marked	
	per cent								
Streptomycin plus PAS									
4 weeks	100.0	—	3.3	22.1	72.1	2.2	0.3	—	—
8 weeks	100.0	0.2	8.0	32.9	53.3	3.5	0.5	—	1.6
12 weeks	100.0	1.1	14.2	36.0	43.0	2.4	1.5	0.2	1.6
16 weeks	100.0	3.3	19.1	35.4	35.6	3.1	1.5	0.4	1.6
20 weeks	100.0	5.2	25.2	34.6	28.5	2.8	1.5	0.6	1.6
24 weeks	100.0	6.9	29.9	29.3	26.3	3.0	2.2	0.8	1.6
28 weeks	100.0	11.9	31.6	25.9	22.9	2.5	3.0	0.6	1.6
32 weeks	100.0	15.7	31.6	23.4	22.0	2.3	2.3	1.1	1.6
36 weeks	100.0	18.7	29.4	22.9	19.9	2.7	2.7	0.8	2.9
40 weeks	100.0	22.1	28.3	20.7	19.2	3.1	2.7	1.0	2.9
Streptomycin plus isoniazid									
4 weeks	100.0	—	3.9	23.6	70.8	1.0	0.5	0.2	—
8 weeks	100.0	1.2	13.2	34.5	48.7	1.4	0.7	0.3	—
12 weeks	100.0	3.2	20.8	36.0	36.5	2.1	1.4	—	—
16 weeks	100.0	7.7	26.9	30.5	31.2	1.6	1.6	—	0.5
20 weeks	100.0	11.4	31.1	26.6	26.9	1.4	1.6	—	1.0
24 weeks	100.0	15.4	31.8	24.1	22.5	2.1	2.2	0.3	1.6
28 weeks	100.0	19.1	31.6	22.6	19.4	2.1	2.4	0.7	2.1
32 weeks	100.0	23.8	31.1	19.1	19.3	1.6	2.3	0.7	2.1
36 weeks	100.0	27.0	29.1	20.3	17.1	1.6	1.9	0.9	2.1
40 weeks	100.0	30.4	28.6	18.9	15.7	1.8	1.8	0.7	2.1
Isoniazid									
4 weeks	100.0	—	4.4	23.1	69.7	1.8	0.5	—	0.5
8 weeks	100.0	1.0	9.8	33.3	53.3	1.5	0.6	—	0.5
12 weeks	100.0	2.6	14.1	39.2	39.0	3.6	1.0	—	0.5
16 weeks	100.0	4.7	20.8	36.6	32.2	3.3	1.7	0.2	0.5
20 weeks	100.0	7.4	26.5	32.1	27.6	3.5	1.7	0.7	0.5
24 weeks	100.0	10.5	29.1	28.1	25.2	3.3	2.6	0.7	0.5
28 weeks	100.0	13.0	30.1	27.0	22.7	2.6	2.6	1.0	1.0
32 weeks	100.0	16.9	29.0	23.9	21.7	3.0	3.7	0.8	1.0
36 weeks	100.0	20.9	28.2	21.0	20.1	3.4	4.1	0.8	1.5
40 weeks	100.0	24.2	26.4	19.6	20.1	2.9	3.4	1.4	2.0

* Excludes 4 nontuberculous deaths in the streptomycin plus isoniazid group.

advanced disease without evidence of cavitation; and only a few (4 per cent), minimal involvement.

Deaths. Thirteen patients died of tuberculosis during the forty-week period, 5 who

had received streptomycin-PAS, 4 who had received streptomycin-isoniazid, and 4 who had received isoniazid.

Changes in roentgenographic appearance: The data on roentgenographic change for this report have been obtained from the fourth evaluation conference of the investigators held in April, 1953. As in the three preceding conferences, all of the films for each patient have been judged independently by three physicians without any knowledge of the patient's identity, clinical condition, or treatment. The percentage distribution of the resulting opinions of roentgenographic change between selection and each subsequent four-week period is presented in table 1 for each regimen.

On all three regimens, two opposite trends may be observed, one toward improvement, the other toward deterioration.

The trend in the direction of improvement was very pronounced on all regimens. The highest percentage in the category of "slight" improvement was reached in the twelfth week; in the category of "moderate" improvement, in the twenty-eighth week; but the percentage in the "markedly" improved group continued to increase through the fortieth week. Evidently the movement of patients from the "no change" group into the improved categories does not keep pace with the shift toward greater improvement once patients have begun to improve. By the fortieth week, some degree of improvement was observed in the films of 78 per cent of the streptomycin-isoniazid patients, 71 per cent of the streptomycin-PAS patients, and 70 per cent of the isoniazid patients.

When significant improvement is restricted to the "marked" and "moderate" categories and they are combined as in the following table, the streptomycin-isoniazid group shows a slight but consistent superiority throughout treatment while no difference is discernible between the other two regimens.

PROPORTION OF EACH GROUP SHOWING MARKED OR MODERATE ROENTGENOGRAPHIC IMPROVEMENT

REGIMEN	WEEKS OF TREATMENT									
	4	8	12	16	20	24	28	32	36	40
Streptomycin-PAS	3.3	8.2	15.3	22.4	30.4	36.8	43.5	47.3	48.1	50.4
Streptomycin - Isoniazid	3.9	14.4	24.0	34.6	42.5	47.2	50.7	54.9	56.1	59.0
Isoniazid	4.4	10.8	16.7	25.5	33.9	39.6	43.1	45.9	49.1	50.6

The tendency of patients to become worse, roentgenographically, was very much less than the tendency to improve. Less than 10 per cent of the total group have shown any deterioration or died of tuberculosis. If "slight deterioration" is disregarded, only 7 per cent of the streptomycin-PAS and isoniazid groups and 5 per cent of streptomycin-isoniazid group grew worse during the forty-week treatment period.

Changes in sputum, fever, and body weight: In table 2, the proportion of patients on each regimen who were in an unfavorable condition with respect to important signs of tuberculous disease is shown for each four-week period.

The bacterial content of sputum specimens was determined both by direct examination and by culture. A consistently higher proportion of specimens positive for *M. tuberculosis* was detected by culture. At the end of treatment, sputum cultures positive for tubercle bacilli were obtained from approximately two-tenths of the streptomycin-isoniazid group and from approximately three-tenths of the patients on the other two regimens.

TABLE 2

PROPORTION OF 583 PATIENTS WITH STREPTOMYCIN-SUSCEPTIBLE INFECTIONS IN UNFAVORABLE CLINICAL CONDITION

NUMBER OF CASES	STREPTOMYCIN-PAS		STREPTOMYCIN-ISONIAZID		ISONIAZID	
	183		195		205	
Per Cent with Sputum Positive for Tubercle Bacilli						
	By Direct Examination	By Culture	By Direct Examination	By Culture	By Direct Examination	By Culture
Selection	63.4	73.8	60.0	69.7	54.6	71.7
4 weeks	47.5	55.7	32.1	52.8	30.7	49.3
8 weeks	39.9	42.6	24.6	38.5	22.5	36.6
12 weeks	31.7	32.8	17.4	25.1	22.9	34.1
16 weeks	27.3	33.9	14.3	19.0	24.2	33.2
20 weeks	27.3	33.9	13.4	17.4	25.6	39.0
24 weeks	21.4	33.3	15.9	21.5	23.1	37.1
28 weeks	24.2	32.8	13.4	18.5	27.7	32.2
32 weeks	23.5	37.2	11.8	15.9	24.9	35.1
36 weeks	28.9	29.5	12.4	19.5	23.9	32.7
40 weeks	27.7	32.2	15.0	17.4	24.4	28.8
Per Cent with Copious Sputum						
Selection	22.4		20.5		20.5	
4 weeks	22.0		21.6		20.3	
8 weeks	23.5		17.5		20.6	
12 weeks	18.7		10.3		14.4	
16 weeks	16.5		7.2		12.9	
20 weeks	20.9		7.7		12.9	
24 weeks	17.0		10.8		11.9	
28 weeks	16.4		10.4		11.6	
32 weeks	14.7		9.8		12.1	
36 weeks	18.0		8.8		8.4	
40 weeks	14.8		6.2		10.7	
Per Cent Febrile*						
Selection	20.2		22.1		20.5	
4 weeks	13.7		11.8		15.1	
8 weeks	15.3		14.9		16.6	
12 weeks	14.2		9.2		10.8	
16 weeks	12.5		10.2		14.7	
20 weeks	10.9		8.7		9.3	
24 weeks	10.9		8.7		8.8	
28 weeks	9.8		7.8		6.9	
32 weeks	9.3		6.7		8.4	
36 weeks	11.5		6.7		7.3	
40 weeks	10.5		6.7		9.3	
Per Cent Underweight†						
Selection	47.5		50.0		55.2	
4 weeks	45.3		47.4		48.1	
8 weeks	44.2		41.1		44.6	
12 weeks	41.0		37.2		42.7	
16 weeks	39.9		35.8		40.5	
20 weeks	37.7		34.2		38.7	
24 weeks	36.3		31.9		36.3	
28 weeks	35.3		30.2		34.1	
32 weeks	34.1		28.7		33.5	
36 weeks	34.8		29.1		33.1	
40 weeks	34.0		28.8		32.8	

* Median of highest oral temperature for five days: febrile 99.0°F. and over.

† Minus deviation of more than 10 per cent from standard height-weight values.

The measurement of the volume of sputum is obviously subject to great variations and the results can furnish only a crude indication of the results of treatment. While the proportion of patients producing copious amounts of sputum decreased on all regimens during the early weeks of treatment, little change has occurred since the sixteenth week.

TABLE 3

RESULT OF CULTURE EXAMINATIONS FOR M. TUBERCULOSIS AT TWENTY-EIGHT AND FORTY WEEKS

28 WEEKS	40 WEEKS						
	Total	Positive	Negative	No Sputum	Contaminated	Not Available	Dead
Streptomycin plus PAS							
Total	183	54	117	1	2	4	5
Positive	57	44	10	—	—	1	2
Negative	115	7	104	1	2	1	—
No sputum	2	1	1	—	—	—	—
Contaminated	4	2	2	—	—	—	—
Not available	2	—	—	—	—	2	—
Dead	3	—	—	—	—	—	3
Streptomycin plus isoniazid							
Total	195	30	155	2	—	4	4
Positive	32	24	8	—	—	—	—
Negative	149	6	142	1	—	—	—
No sputum	3	—	2	1	—	—	—
Contaminated	3	—	3	—	—	—	—
Not available	4	—	—	—	—	4	—
Dead	4	—	—	—	—	—	4
Isoniazid							
Total	205	55	133	4	4	5	4
Positive	64	49	13	—	—	—	2
Negative	130	6	116	4	3	1	—
No sputum	2	—	1	—	1	—	—
Contaminated	3	—	3	—	—	—	—
Not available	4	—	—	—	—	4	—
Dead	2	—	—	—	—	—	2

At most periods, the results with streptomycin-isoniazid were superior to the results with the other two regimens.

On all regimens, the proportion of patients with temperatures above 99° F. decreased markedly during treatment. The decrease was greatest among those receiving streptomycin-isoniazid.

Comparison of the three regimens shows them to be about equal with respect to gain in body weight for those patients who were at least 10 per cent underweight at selection.

Bacteriologic relapse: The question of bacteriologic relapse during treatment has been examined in table 3 by correlating the results of culture examinations at twenty-eight weeks with the results at forty weeks. Among patients whose cultures were negative at twenty-eight weeks, 6 per cent of those receiving streptomycin-PAS, 4 per cent of those receiving streptomycin-isoniazid, and 5 per cent of those receiving isoniazid alone had cultures positive for tubercle bacilli at the fortieth week. Expressed in terms of the total group, cultures were negative for tubercle bacilli at twenty-eight weeks and positive at forty weeks for 4 per cent of the 183 patients who were receiving streptomycin-PAS, 3 per cent of 195 streptomycin-isoniazid patients, and 3 per cent of 205 isoniazid patients. On either basis, the percentages are small and the differences among the three regimens are unimportant.

In summary: Among 583 patients with streptomycin-susceptible infections treated for forty weeks with streptomycin-PAS, streptomycin-isoniazid, or isoniazid alone, those receiving streptomycin-isoniazid maintained a small but consistent advantage on the basis of roentgenographic, clinical, and laboratory findings. On all three regimens, little further gain in the "conversion" of sputum occurred after the twentieth week, but improvement discernible by roentgenogram continued throughout treatment, although at a slower rate during the later weeks.

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SIDE EFFECTS ACCOMPANYING USE OF IPRONIAZID[1]

The observations which follow are based on an extremely limited experience with 32 patients treated with isoniazid[2] and with 27 patients treated with its isopropyl derivative, iproniazid.[2] Similar daily dosage was employed for both drugs, viz., 4 mg. per kg. of body weight. The side effects encountered in the iproniazid-treated group have been so much more varied in character and so much more frequent and severe that even this limited experience has indicated that a clear distinction must be made between these two drugs with regard to their side effects. While observed by many other workers, this distinction was not clearly drawn or emphasized in some of the earlier reports.

Quite early in the experience at Laurel Heights, it became apparent that great improvement in sense of well-being, ravenous appetite, and marked and rapid weight gain occurred with remarkable regularity in the patients who received iproniazid. Similar symptomatic response was far less frequent and impressive in patients who received isoniazid. In the patients who received iproniazid this symptomatic response was frequently out of all proportion to objective improvement as determined by roentgenographic or bacteriologic studies observed either at the time or subsequently. When iproniazid was discontinued, reversal of this symptomatic trend was usually so prompt and pronounced as to constitute a part of the "withdrawal syndrome" to be described below. It appears, therefore, that even the initial favorable symptomatic response to iproniazid may actually be, in part at least, a side effect of the drug. Certainly it is achieved at a considerable price in terms of other risks.

The well-known and regularly reported toxic effects, such as hyperreflexia, muscle twitching, constipation, etcetera, were observed in both groups of patients. In the 32 patients who received isoniazid, these were the only untoward side effects noted, and they were generally mild and of relatively brief duration. In no isoniazid-treated patient did side reactions require cessation of treatment.

In the 27 patients who received iproniazid, the side reactions mentioned were both more frequent and more severe. In addition, a number of far more disturbing toxic manifestations were observed in the iproniazid-treated group. These were as follows:

1. Three patients developed frank psychoses after five to eight weeks of treatment. While all of the 3 patients had abnormal personalities which might be termed pre-psychotic prior to treatment, there seems little doubt that the drug played a role. One patient recovered five weeks after stopping iproniazid; the other 2 continue to require hospitalization in a mental hospital. A disturbing incidence of psychoses in patients treated with iproniazid has also been noted in other institutions. For example, the data obtained by personal communication from Dr. George Wilson, Superintendent and Medical Director at the Uncas-on-Thames Sanatorium at Norwich, Connecticut, and from Dr. P. M. de la Vergne, Assistant Superintendent at the Undercliff Sanatorium at Meriden, Connecticut, are listed in table 1.

2. Two additional patients (of 27 patients) were unable to continue treatment; one because of the appearance of progressive hemolytic anemia, one because of the development of orthostatic hypotension with syncope. Both patients recovered when

[1] Presented in part at the Twelfth Veterans Administration–Army–Navy Conference on the Chemotherapy of Tuberculosis, February 9, 1953, at Atlanta, Georgia.

[2] Isoniazid and iproniazid were supplied through the courtesy of Hoffman-LaRoche, Inc., as Rimifon and Marsilid.

TABLE 1

IPRONIAZID SIDE EFFECTS

SANATORIUM	PATIENTS	PSYCHOSES	RECOVERED
Laurel Heights	27	3	1
Uncas-on-Thames	6	1	1
Undercliff	11	2*	2
Total	44	6	4

* One additional patient had epileptic seizures.

treatment was changed to streptomycin and para-aminosalicylic acid. The major side effects and withdrawal symptoms are shown in the following table.

Iproniazid—4 mg. per kg.
27 Patients

Major side effects—5
3 psychoses (manic 1; paranoid 2)
1 orthostatic hypotension with syncope
1 hemolytic anemia

Withdrawal symptoms
12 patients discontinued treatment
3 patients (<1 month of treatment) = no symptoms
9 patients (6 months of treatment) = 5 severe
4 moderate

3. Among 12 other patients in whom iproniazid treatment was terminated, marked withdrawal symptoms were noted in 9 patients treated for six months; none in 3 patients treated for one month or less. Similar experience has recently been reported by Selikoff, Robitzek, and Ornstein.[3] Symptoms consisted of vivid terrifying dreams, head consciousness, nervousness, restlessness, depression, nausea, and vomiting. Even when dosage was tapered off during seven to ten days, less severe symptoms occurred when a daily level of 50 to 100 mg. was reached. Duration of symptoms was two to four weeks. Phenobarbital in daily doses of 0.045 gm. to 0.075 gm. minimized but did not abolish symptoms.

Neither psychoses nor withdrawal symptoms were noted in any of the 32 patients who received isoniazid.

4. Miscellaneous side effects such as pyrabenzamine hypersensitivity (excitation), pontocaine hypersensitivity (excitation), and angioneurotic edema were noted in individual patients. One 40-year-old patient, after gaining 60 pounds during five months of treatment, developed glycosuria and hyperglycemia with a diabetic-type glucose tolerance curve. Iproniazid was continued plus small doses of insulin, and within two months glucose tolerance curves were normal.

[3] SELIKOFF, I. J., ROBITZEK, E. H., AND ORNSTEIN, G. C.: Am. Rev. Tuberc., 1953, *67*, 212.

In view of the high incidence of undesirable side effects, the use of iproniazid has been discontinued at Laurel Heights except for the few remaining patients who are currently under treatment. In certain well-selected patients, its use under constant medical supervision in an institution may occasionally be justified, but the need for extreme caution is obvious. There appears to be little doubt that the risk of serious toxicity is much greater with iproniazid than with isoniazid and that the latter can be used with far greater safety.

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THE EFFECT OF KETONE COMPOUNDS ON THE INHIBITION OF GROWTH OF TUBERCLE BACILLI BY ISONIAZID IN VITRO

During a search for substances which would antagonize the inhibitory effect of isoniazid on the growth of tubercle bacilli, sodium pyruvate and other keto-compounds were found to have such an effect. This is shown in the following experiments.

Tween-albumin medium was inoculated with 1/100 of its volume of a seven-day-old culture of tubercle bacilli. The suspension was distributed in aliquots of 5.4 ml. into sterile culture tubes. Isoniazid was added to these tubes in decreasing concentrations in order to produce final concentrations of isoniazid varying from 5 to 0.02 γ per ml. Isoniazid was omitted from the last tube of each series. To each series of tubes various amounts of pyruvate were added, one series receiving distilled water instead of pyruvate. The total volume in all tubes was 6 ml. The tubes were incubated at 37° C. and growth was followed by density measurements. The results of these experiments are summarized in table 1, in which the minimal concentrations of isoniazid which inhibited the growth of tubercle bacilli are indicated for media with various amounts of pyruvate and for media without pyruvate. The weight ratio of pyruvate and isoniazid in these tubes is also indicated in parentheses.

It can be seen from this table that the minimal inhibiting concentration of isoniazid increased roughly proportionately with the concentration of pyruvate. The minimal inhibiting concentration of isoniazid, with several strains, was approximately 1 γ per ml. when pyruvate was present in a concentration of 5 mg. per ml., i.e., twenty times higher than in the absence of pyruvate. Pyruvate showed a similar effect when incorporated in agar medium containing isoniazid (table 2). It should be noted that pyruvate exerted a similar effect when isoniazid-resistant organisms were used; these bacilli grew in even higher concentrations when pyruvate was included in the medium. Concentrations of pyruvate higher than 5 mg. per ml. could not be used because of their toxic effect.

A number of substances other than pyruvate were also tested for their antagonistic effect on isoniazid. Ketoglutarate was found to have the same effect as pyruvate. Acetone, when added in an initial concentration of 5 mg. per ml., had a smaller but definite antagonistic effect. Several non-ketone substances known to be metabolized (glutamate, succinate, fumarate, lactate) and several growth factors (pyridoxine hydrochloride, pyridoxal hydrochloride, nicotinic acid, nicotinamide, riboflavin, and PABA) had no effect. These observations suggest that only substances containing a ketone group are able to neutralize the antituberculous effect of isoniazid. This neutralizing effect might be attributed to the known property of the hydrazine group of isoniazid to react with ketone compounds under certain conditions. It is, however, not clear if such a reaction can take place under the conditions existing in the culture medium.

As may be seen in table 1, the pyruvate-isoniazid ratio was markedly different for isoniazid-resistant organisms as compared with that obtained for drug-susceptible bacilli. This suggests that the bacteria may be involved in the mechanism by which pyruvate antagonizes the effect of isoniazid and that this neutralization is not simply the result of a chemical reaction between these two agents. One possible hypothesis to explain the observations is that the bacilli metabolize pyruvate[1-5] and form another keto-compound

[1] Schaefer, W. B.: Ann. Inst. Pasteur, 1948, *74*, 458.
[2] Youmans, A. S., and Youmans, G. P.: J. Bact., 1953, *65*, 96.
[3] Youmans, A. S., and Youmans, G. P.: J. Bact., 1953, *65*, 100.
[4] Boissevain, C. H.: Proc. Soc. Exper. Biol. & Med., 1943, *54*, 344.
[5] Schaefer, W. B.: J. Exper. Med., 1952, *96*, 207.

TABLE 1

MINIMAL CONCENTRATIONS OF ISONIAZID (γ PER ML.) WHICH INHIBITED THE GROWTH OF TUBERCLE BACILLI IN A TWEEN-ALBUMIN MEDIUM CONTAINING VARIOUS CONCENTRATIONS OF SODIUM PYRUVATE

STRAINS TESTED	CONCENTRATIONS OF SODIUM PYRUVATE ADDED (MG. PER ML.)						
	0	0.15	0.3	0.5	1.0	2.5	5.0
H37Rv	0.025	0.08 (2,000)*		0.10 (5,000)	0.10 (10,000)	0.25 (10,000)	0.50 (10,000)
H37Rv	0.05			0.25 (2,000)	0.25 (2,000)	0.5 (5,000)	1.00 (5,000)
B. Vallée	0.04	0.08 (2,000)	0.15 (2,000)	0.25 (2,000)	0.25 (4,000)	0.62 (4,000)	1.25 (4,000)
BCG	0.05				0.50 (2,000)	1.00 (2,500)	2.50 (2,000)
B. dysg. 1	0.08	0.15 (1,000)	0.30 (1,000)		0.50 (2,000)	1.25 (2,000)	2.50 (2,000)
B. dysg. 1	0.05			0.25 (2,000)	0.25 (4,000)	0.50 (5,000)	1.00 (5,000)
B. dysg. 2	0.04					0.62 (4,000)	
H37Ra	0.05						1.00 (5,000)
B. Vallée resistant	100.0					1,000 (2.5)	
BCG resistant	250.0				1,000 (1)		
B. dysg. 1 resistant	50.0					500 (5.0)	
B. dysg. 2 resistant	100.0					1,000 (2.5)	
Avian	1.0					25.00 (200)	

* The figures in parentheses indicate the weight ration sodium pyruvate/isoniazid in the corresponding tube.

which may react with the drug and thus prevent isoniazid from interfering with essential reactions.[6]

[6] SCHAEFER, W. B.: The effect of isoniazid on growing and resting tubercle bacilli (to be published).

Pyruvate antagonized the antituberculous action, not only of isoniazid, but also of β-resorcylic-acid-hydrazid (Dougherty)[7] (minimal inhibiting concentrations 5 γ per ml. in the absence, and 100 γ per ml. in the presence, of 5 mg. of pyruvate per ml.). It also reversed the action of semicarbazide (minimal inhibiting dose 0.1 to 0.25 mg. per ml. in the absence, and 2.5 mg. per ml. in the presence, of 5 mg. of pyruvate per ml.) and that of hydroxylamine (minimal inhibiting dose 80 γ per ml. in the absence, and 600 γ per ml. in the presence, of 1 mg. of pyruvate per ml.). All of these compounds are known to react with ketone compounds under certain conditions. Pyruvate, on the contrary, had no effect on the antituberculous properties of streptomycin or spermine[8] or *p*-acetaminobenzalthiosemicarbazone (amithiozone).[9] Favour[10] reported the inhibitory effect of carboxymethoxylamine hemi-chloride on a variety of bacteria and its reversal by pyruvate and

TABLE 2

GROWTH OF TUBERCLE BACILLI ON AGAR MEDIUM WITH VARIOUS AMOUNTS OF ISONIAZID AND WITH OR WITHOUT SODIUM PYRUVATE (5 MG. PER ML.)

ISONIAZID (γ per ml.)	NO PYRUVATE ADDED			PYRUVATE ADDED		
	10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
Strain *B.* Vallée on Oleic-Acid–Albumin Agar						
1.0	0	0	0	∞	∞	48
0.5	0	0	0	∞	∞	45
0.25	0	0	0	∞	∞	70
0.1	34	5	2	∞	∞	46
0.0	∞	∞	110	∞	∞	59
Strain BCG on Albumin Agar						
1.0	3	1	0	100	15	0
0.5	1	0	0	∞	∞	70
0.25	0	0	0	∞	∞	82
0.0	∞	∞	85	∞	∞	107

acetone. Dewey, Kidder, and Parks[11] have shown that pyruvate and ketoglutarate antagonize the inhibitory action of semicarbazide and of thiosemicarbazides on the growth of Tetrahymena geleei. These investigators attributed these effects to the chemical interaction between the keto-compounds and the inhibitory substances. Future experiments will show if the addition of pyruvate to the culture medium facilitates the isolation of tubercle bacilli from patients under treatment with isoniazid.

[7] Kindly supplied by Dr. René J. Dubos.

[8] HIRSCH, J. G., AND DUBOS, R. J.: J. Exper. Med., 1952, *95*, 191.

[9] Kindly supplied as Tibione by Schenley Laboratories, Inc., New York.

[10] FAVOUR, C. B.: J. Bact., 1948, *55*, 1.

[11] DEWEY, V. C., KIDDER, G. W., AND PARKS, R. E., JR.: J. Gen. Microbiol., 1952, *6*, 181.

In summary: Sodium pyruvate, ketoglutarate, and acetone counteracted the inhibitory effect of isoniazid on the growth of tubercle bacilli *in vitro.*

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ANTAGONISM OF ISONIAZID AND STREPTOMYCIN IN EXPERIMENTAL INFECTION OF MICE WITH M. TUBERCULOSIS H37RV

The combination of isoniazid and streptomycin was first suggested by Szybalski and Bryson[1] on the basis of *in vitro* experiments with *M. ranae* as a means of reducing the frequency of emergence of isoniazid-resistant mutants. In experiments *in vitro* with *M. tuberculosis*, Rist[2] claimed to have prevented drug resistance by combining isoniazid and streptomycin, while Ilavsky[3] reported that these two drugs exhibited a synergistic effect, an observation which has also been described by Garattini and associates on the basis of experiments in mice.[4] More recently, however, Szybalski and Bryson[5] observed the interesting phenomenon that, in *in vitro* tests with *M. ranae*, subinhibitory concentrations of streptomycin antagonized the activity of isoniazid. Similar observations in animals infected with *M. tuberculosis* are presented in this note.

The technique of the present experiments, which employed mice infected intravenously with *M. tuberculosis* H37Rv, has been described earlier.[6] The animals which were to receive isoniazid were allowed to feed ad libitum on a medicated diet so that they received approximately 1.5 to 10 mg. per kg. per day of the substance. Those animals which received combined therapy were, in addition, injected once daily with graded doses of streptomycin ranging from 5 to 300 mg. per kg. Groups of animals which received either isoniazid or streptomycin alone in the same dosage ranges were included in these experiments.

All of the animals were treated and observed for a period of twenty-one days, sacrificed on the twenty-second day, and the lungs were examined for the presence or absence of gross tuberculous lesions. The results of the present experiments are given in table 1.

To consider these data it seems best first to examine the results which were observed with streptomycin or isoniazid alone. It can be seen from *Group E* (table 1) that 50 mg. or more of streptomycin per kg. daily prevented the appearance of lesions in 70 per cent or more of the animals, while doses of 25 mg. per kg. per day or less were ineffective. With isoniazid, 5 mg. per kg. per day (*Group C*) may be considered as the effective dose in that it protected 90 per cent of the animals, while 3.1 mg. per kg. per day (*Group B*) were without activity. These results are in agreement with earlier work.[7]

The examination of the data of combination therapy, as given in table 1 allows four conclusions to be made:

Inactive doses of streptomycin inhibited the activity of an active dose of isoniazid. This may be seen in *Group C*, in which 5 and 15 mg. of streptomycin per kg. daily inhibited the activity of 5 mg. of isoniazid per kg. daily.

Inactive doses of isoniazid were able to inhibit active doses of streptomycin. Examples of this can be seen in *Group A*, in which 1.5 mg. of isoniazid per kg. daily exerted an antagonistic effect on the normally active doses of 50 and 150 mg. of streptomycin

[1] Szybalski, W., and Bryson, B.: Am. Rev. Tuberc., 1952, *65*, 768.

[2] Rist, N.: Presse méd., 1952, *60*, 806.

[3] Ilavsky, J.: Am. Rev. Tuberc., 1952, *65*, 777.

[4] Garattini, S., Grassi, C., Mantegazza, P., Morvillo, V., Tommasini, R., and Trabucchi, E.: Atti Soc. Lombarda Sc. med. e biol., 1952, *7*, 1.

[5] Szybalski, W., and Bryson, B.: Abstract 112 Meeting Am. Chem. Soc., Div. of Med. Chem., 1952, p. 211.

[6] Grunberg, E., and Leiwant, B.: Proc. Soc. Exper. Biol. & Med., 1951, *77*, 47.

[7] Grunberg, E., and Schnitzer, R. J.: Quart. Bull. Sea View Hosp., 1952, *13*, 3.

per kg. daily, and in *Group B*, in which 3.1 mg. of isoniazid per kg. daily inhibited the activity of 50 mg. of streptomycin per kg. daily.

If sufficiently active amounts of one of the substances were given, no inhibition was seen. Examples of this may be observed in *Group D*, in which the effective dose of 10 mg. of isoniazid per kg. daily was used; and in *Groups A* and *B*, in which the effective dose of 300 mg. of streptomycin per kg. daily and the subeffective doses of 1.5 and 3.1 mg. of isoniazid per kg. daily were used and found to give the activity which was to be expected of an adequate dose of one active agent.

No additive effect seemed to occur between the two components. This seems to be true from *Groups B* and *C*, in which, using 25 mg. of streptomycin in combination with

TABLE 1

ANTAGONISM OF ISONIAZID AND STREPTOMYCIN IN THE EXPERIMENTAL TUBERCULOSIS INFECTION OF WHITE MICE

Infection: 0.5 cc of a 10^{-4} dilution of a 7- to 10-day-old Dubos culture of *M. tuberculosis* H37Rv
Duration of treatment: 21 days
Autopsy: twenty-second day

GROUP	ISONIAZID	STREPTOMYCIN	PROTECTION RATE*	REMARKS
	mg/kg/day	*mg/kg/day*		
A	1.5	0.0	0/20 (0%)	
	1.5	25.0	1/10 (10%)	
	1.5	50.0	0/10 (0%)	Inhibition
	1.5	150.0	4/10 (40%)	Inhibition
	1.5	300.0	10/10 (100%)	
B	3.1	0.0	1/19 (5.3%)	
	3.1	25.0	2/10 (20%)	
	3.1	50.0	11/40 (27.5%)	Inhibition
	3.1	150.0	7/10 (70%)	
	3.1	300.0	10/10 (100%)	
C	5.0	0.0	18/20 (90%)	
	5.0	5.0	2/20 (10%)	Inhibition
	5.0	15.0	13/29 (44.8%)	Inhibition
	5.0	25.0	9/10 (90%)	
	5.0	50.0	10/10 (100%)	
D	10.0	0.0	10/10 (100%)	
	10.0	5.0	10/10 (100%)	
	10.0	15.0	10/10 (100%)	
E	10.0	25.0	9/9 (100%)	
	0.0	25.0	0/9 (0%)	
	0.0	50.0	7/10 (70%)	
	0.0	150.0	8/10 (80%)	
F	0.0	0.0	0/49 (0%)	

* Number of animals with no gross lung lesions/number of animals used.

3.1 and 5 mg. of isoniazid per kg. daily, it may be noted that only the effect of the active dose of isoniazid was observed.

These experiments, therefore, seem to indicate that the phenomenon of therapeutic antagonism which has recently been reviewed by Jawetz[8] for quite a number of chemotherapeutically active agents also applies to the combination of streptomycin and isoniazid in tuberculous infections of mice.

It should be particularly emphasized that this antagonism is limited to those situations in which inactive doses of one of the drugs are combined with minimal active doses of the other.

Experiments are in progress to analyze the mechanism of this antagonism. A possible explanation, however, may be that the influence which the subtherapeutic doses of the antagonizing drug exert on the multiplication and normal metabolism of *M. tuberculosis* interferes with the activity of the antagonized agent.[9, 10] This assumption is in part supported by the finding in this laboratory that, in an experimental infection of mice with *E. coli* which does not respond to isoniazid, an oral dose of this drug (25 mg. per kg. per day) did not antagonize active doses of streptomycin of 100 to 300 mg. per kg. per day.

In summary: Mutual antagonism of isoniazid and streptomycin was demonstrated in the experimental infection of mice with *M. tuberculosis* H37Rv if subtherapeutic doses of either drug were combined with minimal active doses of isoniazid or streptomycin, respectively.

Although the present writers are aware that the experimental conditions maintained in this study might not be paralleled in clinical dosage schedules, it seems appropriate to suggest the administration of fully active doses of streptomycin and isoniazid in combined therapy of human tuberculosis.

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[8] JAWETZ, E.: Arch. Int. Med., 1952, *90*, 301.
[9] GUNNISON, J. B., SPECK, R. S., JAWETZ, E., AND BRUFF, J. A.: Antibiotics, 1951, *1*, 259.
[10] EAGLE, H.: Am. J. Med., 1952, *13*, 389.

CONDITIONAL ANTAGONISM BETWEEN ISONIAZID AND OTHER ANTIBACTERIAL AGENTS[1]

As previously reported, *Mycobacterium ranae* is inhibited by 5 γ of isoniazid per ml. when plated on a supplemented nutrient agar.[2] A small percentage of the cells, however, is almost one thousand times as resistant to this chemotherapeutic agent. In a search for synergistic drugs, it was observed that subinhibitory concentrations of streptomycin antagonize the action of isoniazid.[3] Thus, on a plate containing 100 γ of isoniazid per ml. and seeded with 10^6 to 10^7 cells of *M. ranae*, growth of the majority of cells is not perceptible, with 10^{-3} to 10^{-4} per cent of the bacteria developing resistant colonies. If 0.3 γ of streptomycin per ml. is added to such a plate (the inhibitory concentration of streptomycin being 0.5 γ per ml.), almost all cells are able to grow and form colonies, although their growth rate is strongly decreased.

These studies have now been extended to include several other antimycobacterial agents, using three saprophytic *Mycobacteria* (*M. ranae*, *M. smegmatis*, and a nonidentified *Mycobacterium sp.*[4]). All plating was done on Difco nutrient agar, supplemented with 2 per cent glycerol and 0.1 per cent yeast extract.

Figure 1 represents the percentage of colony-forming cells in a suspension of *M. ranae* plated in the presence of 100 γ of isoniazid per ml. and increasing concentrations of streptomycin. The three curves represent three strains: the wild type susceptible to 0.5 γ of streptomycin per ml., and two streptomycin-resistant strains, one susceptible to 5 γ per ml. and the other partially inhibited at 25,000 γ per ml., both derived from the wild type.

It may be clearly seen that, within the ranges of approximately 10 to 90 per cent of the inhibitory concentration, streptomycin antagonizes isoniazid action, allowing a higher proportion of cells to form colonies. This antagonism, however, is not complete: the appearance of the colonies is considerably delayed, with prolongation in time of development from the normal two-day period to approximately seven days. Upon emergence, colonies grown in the presence of streptomycin-antagonized isoniazid are composed of more than 50 per cent of isoniazid-resistant cells. Their streptomycin sensitivity is not changed.

If both drugs are present at concentrations well over the inhibitory threshold, the principle of multiple chemotherapy applies and no colonies appear, for the theoretical probability of appearance of doubly resistant mutants is only approximately 10^{-15}, i.e. 10^{-6} (for isoniazid) $\times$ 10^{-9} (for streptomycin). An example of synergism has been reported by Ilavsky[5] wherein low concentrations of streptomycin cause a two- to threefold decrease of the isoniazid inhibitory threshold for streptomycin-sensitive and -resistant *M. tuberculosis*. The use of liquid medium by this investigator, a relatively short period of observation of the assays, and other differences in experimental conditions make it difficult to interpret the results in terms of the present data. In similar experiments, Aitoff and

[1] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[2] Szybalski, W., and Bryson, V.: Am. Rev. Tuberc., 1952, *65*, 768.

[3] Szybalski, W., and Bryson, V.: Abstracts of Papers, 112th Meeting American Chemical Society, Atlantic City, New Jersey, September 14–19, 1952, Div. of Med. Chem., p. 21–L.

[4] Kindly supplied by W. Steenken, Jr. (7th subculture), L. H. Geronimus, and F. Carvajal, respectively.

[5] Ilavsky, J.: Am. Rev. Tuberc., 1952, *65*, 777.

Salas[6] observed a small degree of synergism, but Pansy, Stander, and Donovick[7], on the contrary, reported a slightly less than additive effect.

Several hypotheses may account for the observed antagonism. The simplest assumes a chemical interaction between isoniazid and streptomycin with consequent neutralizing effect as proposed by Berczeller and Berczeller[8] in their study of the effect of subinhibitory concentrations of isoniazid in changing the inhibitory levels of streptomycin for *M. pyogenes* and *E. coli*. This interpretation is, however, highly improbable as an explanation of the present experimental findings. The antagonism is limited only to a narrow range of subinhibitory streptomycin concentrations. It does not depend on the molecular ratio of streptomycin and isoniazid concentrations, which is extremely low, but is a function of the biologic property of the strain, i.e., its streptomycin susceptibility. The development of isoniazid resistance in colonies growing on streptomycin-antagonized isoniazid medium is also contradictory to the theory of simple chemical interaction. An antagonistic action of streptomycin on *p*-aminosalicylic acid (PAS)-inhibited, streptomycin-resistant strains of *E. coli*[9] bears a resemblance to the isoniazid–streptomycin interaction described and may have a similar basis.

Any interpretation of streptomycin-isoniazid antagonism must take into account the fact that the antagonistic effect appears only when one of the two drugs is present within a narrow subinhibitory range. In general terms, Jawetz and Gunnison[10] similarly state that antagonism "is observed only when a bactericidal or therapeutically effective concentration of one drug is added to a relatively inactive concentration of the other."

It was found, both by microscopic observation and by washing bacteria from the surface of the agar with subsequent assay of sensitive and resistant cells, that the drug-susceptible *M. ranae*, plated in the presence of 100 γ of isoniazid per ml., undergoes four to eight divisions before growth is completely inhibited. Under this condition, isoniazid has a marked bactericidal effect. When *M. ranae* is plated on the same isoniazid agar supplemented with antagonistic concentrations of streptomycin (0.3 γ per ml.), cells undergo an almost unlimited number of divisions, proceeding, however, very slowly. Protection of bacteria against the effects of certain antibacterial agents by reduction of the growth rate has been reported by numerous investigators (cf. Rosenblum and Bryson[11]). Subinhibitory concentrations of streptomycin, while slowing down cell activity, apparently protect the bacteria from the killing action of isoniazid, and viable sensitive cells can be recovered from such plates during the first three days of incubation. After this period, the total number of cells increases at a faster rate and these new cells are resistant to isoniazid. Microscopic observation reveals that, on the background of a poor, primary growth, secondary, faster growing colonies develop. These apparently isoniazid-resistant secondary papillae overgrow the primary sensitive colonies. Thus, the action of streptomycin cannot be interpreted as that of a perfect mutagen which converts 100 per cent of the cells to isoniazid-resistant mutants. Spontaneous mutations during primary growth on streptomycin-antagonized isoniazid plates are responsible for the appearance of isoniazid-resistant cells. Subsequent selection within every colony favors the predominance of these resistant mutants. However, the present data on the numbers of sensitive cells, insuf-

[6] Aitoff, M., and Salas, A.: Ann. Inst. Pasteur, 1952, *83*, 273.

[7] Pansy, F., Stander, A., and Donovick, R.: Am. Rev. Tuberc., 1952, *65*, 761.

[8] Berczeller, A., and Berczeller, G.: Quart. Bull. Sea View Hosp., 1953, *14*, 3.

[9] Pratt, R., Divatia, K. J., Gardner, G., and Dufrenoy, J.: J. Am. Pharm. Assoc., 1951, *40*, 155.

[10] Jawetz, E., and Gunnison, J. B.: J. A. M. A., 1952, *150*, 693.

[11] Rosenblum, E. D., and Bryson, V.: Antibiotics and Chemotherapy (in press).

ficiently precise as they are, indicate that the mutation rate to isoniazid resistance increases during bacterial growth on streptomycin-antagonized isoniazid agar.

Experiments performed with two other saprophytic *Mycobacteria* gave very similar results. When subinhibitory concentrations of aureomycin or terramycin were substituted for streptomycin, curves essentially like those of figure 1 were obtained, except that 1 per cent was the highest proportion of cells growing in the range of antagonistic concentrations.

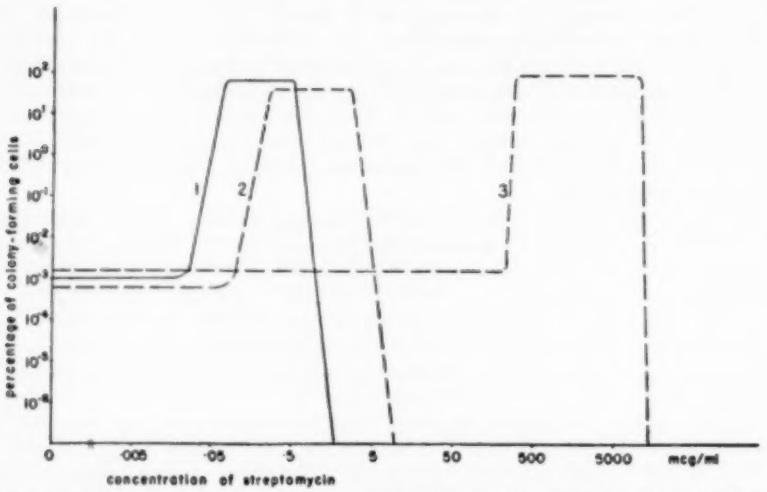

FIG. 1. Isoniazid-streptomycin interaction as studied with three strains of *Mycobacterium ranae*. The curves represent the percentage of cells forming colonies on supplemented nutrient agar containing 100 γ of isoniazid per ml. and varying concentrations of streptomycin. Behavior of the wild-type strain sensitive to 0.5 γ of isoniazid per ml. is depicted by curve 1. Curves 2 and 3 are for resistant strains derived from the first strain with inhibitory thresholds at 5 and 25,000 γ of streptomycin per ml, respectively. Logarithmic scales are used for both axes. Each curve is based on two to ten experiments.

Viomycin, neomycin, PAS, and nicotinaldehyde thiosemicarbazone do not show any antagonistic effect against isoniazid.[13] If present at inhibitory concentrations, they prevent the development of resistant colonies, in agreement with the multiple chemotherapy principle. Further investigation is required to see if these studies carry any practical implications for *in vivo* experiments or for work with pathogenic *Mycobacteria*.

In summary: Streptomycin at a subinhibitory concentration antagonizes the action of isoniazid against streptomycin-susceptible and -resistant saprophytic *Mycobacteria*.

[13] We are indebted to Dr. L. B. Hobson of E. R. Squibb & Sons for streptomycin sulfate; to Dr. H. A. Nelson of the Upjohn Company for neomycin sulfate; to Dr. J. H. Kane and Dr. F. W. Tanner, Jr. of Chas. Pfizer & Co. for isoniazid, terramycin, and viomycin; to Dr. J. A. Aeschlimann and Dr. R. A. Lewis of Hoffmann-LaRoche for nicotinaldehyde thiosemicarbazone hydrochloride and isoniazid; to Lederle Laboratories Division for aureomycin; and to Dr. O. E. Graessle of Merck Institute for sodium-p-amino salicylate (PAS).

When a bacterial suspension is plated in the presence of inhibitory concentrations of isoniazid, only resistant cells develop colonies (10^{-4} to 10^{-3} per cent); addition of streptomycin in concentrations between one-tenth and nine-tenths of its inhibitory level to similar isoniazid plates induces all cells to form isoniazid-resistant, slow-growing colonies. If both drugs are present at concentrations well over the inhibitory threshold, no colonies appear. This antagonism is partly physiologic in origin and is partly due to selection. Experiments show that subinhibitory concentrations of streptomycin (and also aureomycin and terramycin, but not of PAS, viomycin, neomycin, and nicotinaldehyde thiosemicarbazone) slow down the metabolism or reproductive activity of the cells to the level where the cells may multiply only very slowly without being killed by isoniazid. Isoniazid-resistant mutants ultimately appear during this inhibited initial growth and, multiplying more rapidly, outgrow the drug-susceptible bacteria.

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THE INACTIVATION OF ISONIAZID BY DUBOS MEDIUM

The observation of Goulding and his associates[1] that isoniazid is inactivated by "Dubos liquid medium" led to the study of the constituents of Dubos medium to determine the component or components causing this phenomenon.

A Tween 80[2]-serum albumin medium[3] was used in the study. Isoniazid was made up in this medium at a concentration of 200 γ per ml. and incubated at 37°C. in parallel with a control isoniazid solution made up in distilled water. At zero time, 2 days, 5 days, and 7 days the two samples were tested for their activity against *Mycobacterium tuberculosis* var. *bovis* strain BCG in a modified Kirchner's medium.[4] The minimal inhibiting concentrations were determined and are listed in table 1. The data confirm Goulding and his associates[1] in showing inactivation of isoniazid in Dubos medium.

TABLE 1

ACTION OF DUBOS MEDIUM AGAINST ISONIAZID

MEDIUM	MINIMAL INHIBITING CONCENTRATION VS. BCG IN γ PER ML. AFTER			
	0 Days	2 Days	5 Days	7 Days
Dubos	0.0508	0.0838	0.108	0.162
Distilled Water	0.0481	0.0521	0.0556	0.0502

TABLE 2

THE EFFECT OF FERRIC AMMONIUM CITRATE AT VARYING pH'S ON THE INACTIVATION OF ISONIAZID

SOLUTION IN WHICH ISONIAZID (200γ PER ML.) WAS INCUBATED	MINIMAL INHIBITING CONCENTRATION IN γ PER ML. VS. BCG AFTER:	
	3 Days at 37°C.	6 Days at 37°C.
Ferric ammonium citrate plus buffer to pH 4.9	0.0390	0.0525
Ferric ammonium citrate plus buffer to pH 7.0	0.0422	0.0560
Ferric ammonium citrate plus buffer to pH 8.3	0.0583	0.344
Ferric ammonium citrate plus acid to pH 5.0	0.0402	0.0507
Ferric ammonium citrate plus base to pH 7.0	0.0497	0.0466
Ferric ammonium citrate plus base to pH 9.0	0.0520	0.115

The inactivating effect of the various constituents of the Dubos medium on isoniazid was then investigated. In the same manner as above, 4 fractions of the medium, viz., asparagine, Tween 80, serum albumin, and the "salts" consisting of Na_2HPO_4, KH_2PO_4, $Na_3C_6H_5O_7$, $MgSO_4$, and ferric ammonium citrate were tested. Only the "salts" gave an inactivation of the isoniazid.

Further studies revealed that the inactivation took place only with the ferric ammonium citrate when accompanied by the phosphate buffer system. Ferric ammonium

[1] GOULDING, R., KING, M. B., KNOX, R., AND ROBSON, J. M.: Lancet, 1952, *2*, 69.

[2] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[3] DUBOS, R. J., AND MIDDLEBROOK, G.: Am. Rev. Tuberc., 1947, *56*, 334.

[4] DONOVICK, R., PANSY, F., STRYKER, G., AND BERNSTEIN, J.: J. Bact., 1950, *59*, 667.

citrate per se in an aqueous solution produced no inactivation of isoniazid, nor did the "salts" when ferric ammonium citrate was omitted.

Ferric ammonium citrate was made up in varying phosphate buffer solutions at pH 4.9, 7.0, and 8.3. At pH 8.3, inactivation of isoniazid occurred, whereas at pH 4.9 and 7.0 there was no inactivation. Ferric ammonium citrate was then made up in 0.01 per cent solution and the solution was split into three portions. The pH of the three portions was brought to pH 5.0, pH 7.0, and pH 9.0, respectively, by the addition of acid or base. At pH 9.0, inactivation of isoniazid was observed, whereas no inactivation occurred at pH 5.0 or 7.0. The data obtained are shown in table 2.

Ferric chloride 0.01 per cent in distilled water and in phosphate buffer at pH 8.3 was then tested for activity against isoniazid. The aqueous solution gave no inactivation of isoniazid, but at pH 8.3 inactivation was found.

The experiments conducted appear to indicate that the presence of the ferric ion at an alkaline pH will inactivate isoniazid. It is suggested that whenever Dubos medium, or any medium containing the ferric ion, is to be used in the study of isoniazid, the pH of the medium should be adjusted to the acid side. Mitchison[4] has shown that tubercle bacilli will grow well in a Dubos medium adjusted to pH 6.5.

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[4] MITCHISON, D. A.: Lancet, 1952, *2*, 858.

ISONIAZID SERUM CONCENTRATIONS AND TOTAL HEMOGLOBIN AND METHEMOGLOBIN VALUES IN TUBERCULOUS PATIENTS ON TWO DOSAGE REGIMENS OF ISONIAZID THERAPY

Elmendorf, Cawthon, Muschenheim, and McDermott[1] determined weekly isoniazid plasma concentrations during a seven-week period on seven subjects receiving 3 mg. per kg. per day. Their data were obtained on blood samples drawn three hours after the first dose. According to Rubin, Drekter, Scheiner, and DeRitter,[2] the plasma isoniazid concentration reaches its peak at approximately an hour and one-half after oral administration. Robitzek and Selikoff[3, 4] observed their patients (2 to 4 mg. per kg. per day) for alterations in total hemoglobin values weekly, at first, and then every three weeks, but they did not report their results in detail in these two papers nor did they include methemoglobin values. The writers of the present report believed, therefore, that the results of a serial study of simultaneous isoniazid, total hemoglobin, and methemoglobin concentrations as outlined in the following paragraph would be of interest to other investigators and observers concerned with the use of isoniazid in the chemotherapy of tuberculosis.

Nineteen patients who were receiving isoniazid therapy and selected at random were organized into two groups, 10 receiving 50 mg. of the drug three times daily and 9 receiving 100 mg. three times daily. Serial isoniazid serum concentrations and total hemoglobin and methemoglobin values obtained approximately twice weekly during a twelve-week course of isoniazid therapy are presented below.

The blood samples were drawn an hour and one-half after administration of the morning dose, and the isoniazid serum concentrations were determined according to the procedure of Rubin, Drekter, Scheiner, and DeRitter[2] in which isonicotinyl hydrazine is converted to isonicotinic acid and determined colorimetrically at 430 mμ by the reaction with 10 per cent cyanogen bromide in the presence of ammonia buffer, described by Mueller and Fox[5] for nicotinic acid. Blanks were estimated by the reaction with 3 per cent cyanogen bromide and mono-methyl para-aminophenol sulfate (Mallinckrodt Pictol), in which isonicotinic acid yields no color as given by Dann and Handler. The Coleman spectrophotometer, model 6A, was used in these determinations.

Methemoglobin values, obtained on the same sample of blood as the isoniazid serum concentrations, were based on the method of Evelyn and Malloy[7] and determined on the Beckman spectrophotometer, model DU. The calibration factors for this method were obtained by determination of the hemoglobin content of a normal sample of blood by the iron method of Wong.[8]

The isoniazid serum concentrations obtained during this study were examined statistically to determine if the difference in serum concentration on the two dosage regimens constituted a significant variation as related to dosage. The data were also evaluated for

[1] Elmendorf, D. F., Jr., Cawthon, W. U., Muschenheim, C., and McDermott, W.: Am. Rev. Tubere., 1952, *65*, 429.

[2] Rubin, S. H., Drekter, L., Scheiner, J., and De Ritter, E.: Dis. of Chest, 1952, *21*, 439.

[3] Robitzek, E. H., and Selikoff, I. J.: Am. Rev. Tubere., 1952, *65*, 402.

[4] Selikoff, I. J., and Robitzek, E. H.: Dis. of Chest, 1952, *21*, 385.

[5] Mueller, A., and Fox, S. H.: J. Am. Pharm. A., 1951, *15*, 513.

[6] Dann, W. J., and Handler, P.: J. Biol. Chem., 1941, *140*, 201.

[7] Evelyn, K. A., and Malloy, H. T.: J. Biol. Chem., 1938, *126*, 655.

[8] Wong, S. Y.: J. Biol. Chem., 1928, 77, 409.

the extent or degree of inverse correlation of serum concentration with the patient's weight.[9]

Isoniazid serum concentrations for both dosage groups are presented in tables 1 and 2. It is inferred from these data that such a difference in averages between the two groups

TABLE 1

ISONIAZID SERUM LEVELS IN A GROUP OF TUBERCULOSIS PATIENTS RECEIVING 150 MG. PER DAY

PATIENT	WEIGHT	NUMBER OF LEVELS	RANGE	AVERAGE
			γ *per ml.*	γ *per ml.*
G. E.	136	5	1.15–1.93	1.45
L. E.	133	6	1.45–2.67	1.99
R. F.	190	4	1.05–2.48	1.85
B. H.	154	2	2.16–2.57	2.37
R. N.	148	5	1.12–1.83	1.56
H. P.	151	5	1.76–2.88	2.01
C. R.	133	6	1.55–2.59	2.03
C. W.	134	5	1.42–2.70	1.76
H. R.	130	5	1.73–2.60	2.07
T. H.	142	5	0.85–1.79	1.48
Group.....	145 ± 17.9	48	0.85–2.88	1.86 ± 0.296

TABLE 2

ISONIAZID SERUM LEVELS IN A GROUP OF TUBERCULOSIS PATIENTS RECEIVING 300 MG. PER DAY

PATIENT	WEIGHT	NUMBER OF LEVELS	RANGE	AVERAGE
			γ *per ml.*	γ *per ml.*
R. B.	153	2	1.08–3.62	2.35
E. F.	164	5	1.99–2.94	2.47
R. N.	125	4	2.97–4.46	3.83
J. D.	158	5	1.08–2.20	1.70
R. B.	149	5	2.53–2.97	2.74
C. H.	161	3	1.66–2.09	1.84
P. S.	164	5	1.45–2.13	1.72
H. O.	180	5	1.15–2.56	2.03
G. A.	140	5	1.52–4.66	2.91
Group.....	155 ± 15.8	39	1.08–4.66	2.40 ± 0.692

could occur by chance in only 4 per cent of all random samples from a population in which the null hypothesis, i.e., mean of 300 group minus mean of 150 group equals zero, was true ($t = 2.250$). There was apparently no significant difference between the average

[9] The authors would like to express their sincere appreciation to Dr. John H. Vitale and Dr. Roger E. Bardsley for their valuable suggestions in the statistical analysis of our data and to Mr. Albert F. Reed for obtaining our blood samples.

weights of the two groups ($t = 1.284$, per cent; probability level = 22 per cent). The correlation coefficients for isoniazid concentration and body weight may also be readily computed from the data in these tables. These were −0.2, 0.07, and −0.8 for the composite, 150 mg. per day group, and 300 mg. per day group, respectively. The inverse re-

TABLE 3

METHEMOGLOBIN, TOTAL HEMOGLOBIN, AND PER CENT METHEMOGLOBIN VALUES IN A GROUP OF TUBERCULOSIS PATIENTS RECEIVING 150 MG. OF ISONIAZID PER DAY

PATIENT	NUMBER OF SAMPLES	MHb (GM. PER 100 ML.)		TOTAL Hb (GM. PER 100 ML.)		PER CENT MHb	
		Range	Average	Range	Average	Range	Average
G. E.	5	0.0–0.3	0.1	9.4–15.5	13.5	0.0–2.1	0.8
L. E.	5	−0.3–0.3	0.1	12.6–19.9	14.6	−1.4–2.2	0.5
R. F.	4	0.0–0.2	0.1	14.3–17.7	16.2	0.0–1.3	0.8
B. H.	3	0.1–0.1	0.1	13.2–19.1	16.9	0.3–1.1	0.6
R. N.	5	−0.2–0.1	0.0	14.0–20.4	17.9	−1.0–0.8	0.0
H. P.	5	0.0–0.4	0.1	11.8–17.0	13.2	0.1–2.8	1.1
C. R.	6	0.0–0.3	0.1	10.6–12.7	11.9	0.0–2.1	1.0
C. W.	5	0.0–0.1	0.1	12.0–16.4	13.4	0.0–0.8	0.4
H. R.	5	−0.1–0.1	0.0	12.7–18.9	15.0	−0.6–0.5	0.1
T. H.	5	−0.1–0.0	0.0	11.8–19.6	15.9	−1.2–0.0	−0.3
Group..	48	−0.3–0.3	0.1 ± 0.04	9.4–20.4	14.9 ± 1.88	−1.4–2.8	0.5 ± 0.4

TABLE 4

METHEMOGLOBIN, TOTAL HEMOGLOBIN, AND PER CENT METHEMOGLOBIN VALUES IN A GROUP OF TUBERCULOSIS PATIENTS RECEIVING 300 MG. ISONIAZID PER DAY

PATIENT	NUMBER OF SAMPLES	MHb (GM. PER 100 ML.)		TOTAL Hb (GM. PER 100 ML.)		PER CENT MHb	
		Range	Average	Range	Average	Range	Average
R. B.	2	−0.1–0.0	0.0	12.1–21.7	16.2	−0.4–0.0	−0.1
E. F.	5	−0.1–0.1	0.0	12.9–21.3	16.4	−1.1–0.6	−0.1
R. N.	4	0.1–0.2	0.1	15.0–16.4	15.4	0.9–1.1	0.9
J. D.	5	−0.1–0.1	0.0	13.1–18.6	15.5	−1.0–0.6	0.0
R. B.	5	0.0–0.1	0.1	14.3–20.1	16.7	0.0–0.8	0.5
C. H.	3	−0.1–0.1	0.0	12.4–23.0	16.3	−0.2–0.4	0.2
P. S.	5	−0.1–0.1	0.0	11.1–17.1	13.1	−0.4–0.8	0.2
H. O.	5	−0.1–0.2	0.0	12.7–14.1	13.4	−1.1–1.2	0.1
G. A.	5	0.0–0.2	0.1	11.4–13.2	12.2	0.0–1.3	0.7
Group..	39	−0.1–0.2	0.0 ± 0.05	11.1–23.0	15.0 ± 1.67	−1.1–1.3	0.3 ± 0.3

lationship indicated by the coefficient of −0.8 closely approached the perfect correlation of −1 and could occur by chance in only 1 per cent of random samples ($t = 3.536$). This characteristic, peculiar only to the 300 mg. per day group in the present study, permits the inference that the difference between the average isoniazid serum concentrations of these two groups is even more significant than is readily apparent from the data.

In tables 3 and 4 the methemoglobin and total hemoglobin values for both groups are

contained. Van Slyke and his associates[10] give the normal values for per cent methemoglobin as determined by several investigators. Their results vary from 0.0 per cent to 2.4 per cent. Thus, it may be said that only one patient, H. P., in the 150 mg. per day group in the present study showed a definitely high per cent methemoglobin. However, as in the case of others who approached the upper limits of normal, this reverted to normal on subsequent determinations.

The variations in total hemoglobin concentration on 13 of the 19 patients were of sufficient magnitude to result in critical evaluation of the data for a definite trend during the course of chemotherapy. However, no progressive alteration, i.e., either a decrease or increase, was evident as data were examined for the 120-day period of therapy. Three patients in each of the two groups did show generally low total hemoglobins.

No correlations could be drawn between isoniazid concentrations and hemoglobin values.

In summary: Peak isoniazid serum concentrations and total hemoglobin and methemoglobin values were determined for 19 tuberculous patients, 10 receiving 150 mg. per day and 9 receiving 300 mg. per day. These determinations disclosed a group average of 1.86 γ per ml. $\pm$ 0.296 for the 150 mg. per day group as compared with 2.40 γ per ml. $\pm$ 0.692 for the 300 mg. per day group. Statistical analysis revealed a significant difference between these averages. The hemoglobin values revealed one instance of definitely high methemoglobin in the 150 mg. per day group and 3 patients with generally low total hemoglobins in each of the two groups. The abnormality in the patient with the high methemoglobin reverted to normal upon subsequent determinations. No correlations could be drawn between isoniazid concentrations and hemoglobin values.

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[10] VAN SLYKE, D. D., HILLER, A., WEISIGER, J. J., AND CRUZ, W. O.: J. Biol. Chem., 1946, *166*, 121.

VIRULENCE IN GUINEA PIGS OF ISONIAZID-RESISTANT CULTURES ISOLATED FROM CLINICAL SPECIMENS

A Preliminary Report

The determination of virulence of strains of *M. tuberculosis* isolated from clinical material has for a long time been of particular interest to many investigators. The present communication is a preliminary report of a study of virulence in guinea pigs of 25 isoniazid-resistant and 6 isoniazid-susceptible cultures isolated from 31 known tuberculous patients.

The cultures were made on Löwenstein-Jensen[1] and Peizer-Schecter[2] media. Subcultures were made to Proskauer-Beck medium for the preparation of aninal inocula. The cultures in liquid medium were incubated ten days at 37° C. They were then well shaken and diluted with saline until their turbidity matched that of the No. 1 tube of the McFarland scale. Density of organisms was also checked by stained smears prepared from 0.01 ml. of the diluted cultures, spread evenly over an area of one square centimeter on a slide.

Guinea pigs weighing between 200 and 250 gm. were injected subcutaneously into the inguinal region with 0.25 ml. of diluted culture. The animals were tuberculin tested three or four days before and three or four weeks after injection. All animals were re-weighed before autopsy. Ground portions of organs showing gross infection were examined by stained smears and subcultured on solid media.

It may be seen in table 1, that all the animals, whether dead or sacrificed at the end of fifteen weeks, showed some lesions of tuberculosis. It should be noted, however, that typical gross lesions of tuberculous infection in spleen and liver were found only in those which died and in 4 of 18 of those which survived injection of isoniazid-resistant organisms. Tuberculous foci in the spleens of 7 other survivors were discrete, small, and few in number; the livers were negative on gross examination.

Cultures resulting from ground tuberculous tissue from the spleens of these 7 animals were found to be resistant to isoniazid to the same degree as before injection. No other cultures derived from guinea pig lesions were tested for resistance in this trial series. Positive smears and cultures of tubercle bacilli were obtained from homogenized inguinal node tissue in all 31 animals. All of the cultures used in this study were positive by the neutral red test of Dubos and Middlebrook.[3, 4] All animals became tuberculin positive at the end of four weeks.

In summary: It was found that: Isoniazid-resistant tubercle bacilli cause progressive disease in guinea pigs, as indicated by development of positive tuberculin test, lesions containing viable bacilli and, in some cases, death. In this small series, the tuberculous infection caused by resistant strains was less rapidly progressive and less rapidly lethal than the usual infection in guinea pigs in this laboratory. More detailed work is required to study the relationship of virulence and isoniazid resistance of *M. tuberculosis*.

BUREAU OF LABORATORIES
DEPARTMENT OF HEALTH
CITY OF NEW YORK

LENORE R. PEIZER
DANIEL WIDELOCK
SARAH KLEIN

March 4, 1953

[1] JENSEN, K. A.: Zentralbl. f. Bakt., 1932, *125*, 222.
[2] PEIZER, L. R., AND SCHECTER, C.: Am. J. Clin. Path., 1950, *20*, 682.
[3] DUBOS, R., AND MIDDLEBROOK, G.: Am. Rev. Tuberc., 1948, *58*, 598.
[4] DUBOS, R., AND MIDDLEBROOK, G.: Am. Rev. Tuberc., 1949, *60*, 384.

TABLE 1

A Study of the Effect on Guinea Pigs of Injections with Isoniazid-Resistant and Susceptible Strains of M. Tuberculosis from Clinical Material

31 Cultures Injected into an Equal Number of Guinea Pigs

		Number of cultures injected	Number of guinea pigs which died	Number of guinea pigs sacrificed 15 weeks	Number of guinea pigs dead at end of week number						Guinea pigs dead						Guinea pigs sacrificed									Weight of sacrificed guinea pigs at end 15 weeks	
											†	Culture			Smear				Culture			Smear					
											Spleen Liver Node	+ − +	+ + +	− − +	+ − +	+ + +	− − +	Spleen Liver Node	+ − +	+ + +	− − +	+ − +	+ + +	− − +	Number which Gained Weight	Number which Lost Weight
					6	7	8	9	10	12																	
Cultures resistant to:*	100 γ/ml	14	5	9			1	1	1	2		1	4	0	1	4	0		3	2	4	3	2	4	7	2
	50 γ/ml	7	1	6			1					0	1	0	0	1	0		1	2	3	1	2	3	4	2
	25 γ/ml	3	0	3															3	0	0	3	0	0	3	0
	10 γ/ml	1	1	0				1				0	1	0	0	1	0									
Cultures sensitive to:	0.1 γ/ml	5	5	0	1	3	1					0	5	0	0	5	0									
	0.5 γ/ml	1	1	0			1					0	1	0	0	1	0									
Total		31	13	18	1	3	4	2	1	2		1	12	0	1	12	0		7	4	7	7	4	7	14	4

* Resistance was determined by direct inoculation of the concentrated sputum on solid media containing the following amounts of isoniazid: 1 γ per ml.; 0.5 γ per ml.; 1.0 γ per ml.; 5.0 γ per ml. Isolations from primary cultures found to be resistant to 5 γ of the drug per ml. were used to test resistance to 10 γ per ml., 25 γ per ml., 50 γ per ml., 75 γ per ml., and 100 γ per ml. The highest amount of isoniazid per ml. of medium which permitted good growth as compared with the control was accepted as the end point.

† The + sign means positive lesions seen on the organ indicated.

The − sign means no lesions seen.

THE CONTROL OF EXPERIMENTAL MOUSE TUBERCULOSIS BY THE INTERMITTENT ADMINISTRATION OF STREPTOMYCIN, VIOMYCIN, ISONIAZID, AND STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE

The antituberculous activities of streptomycin, isoniazid, and viomycin in both animals and man are well known. The effectiveness of streptomycin and of viomycin, when used intermittently in combination with other antituberculous agents in the treatment of certain forms of tuberculosis in man, furthermore, is established. Neither streptomycin nor viomycin, however, is widely used as a single drug on an intermittent basis, and, in-

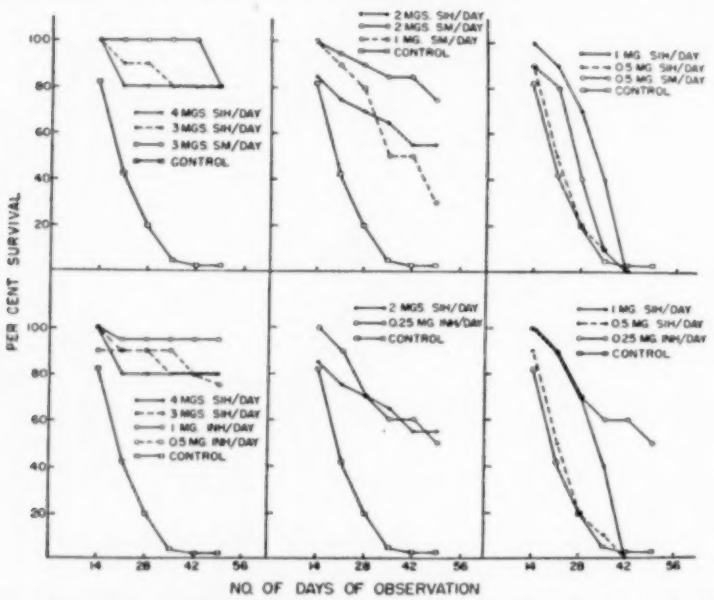

FIG. 1. Comparison of the antituberculous activities of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine when administered twice weekly (Vallée strain: *M. tuberculosis*, bovine).

deed, the observations on isoniazid which have been reported to date have been concerned entirely with its action when administered daily.

Evaluation of the potentialities of intermittent dosage regimens and the use of such intermittent regimens to bring out differences in the mechanisms by which antituberculous agents deport themselves *in vivo* is a laborious and lengthy procedure in man. It seemed of interest, therefore, to compare the effects of streptomycin, viomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine (e.g., streptomycin and isoniazid in chemical combination)[1] in the control of experimental tuberculosis of mice, using intermittent dosage regimens.

[1] HOBBY, G. L., LENERT, T. F., RIVOIRE, Z., DONIKIAN, M., AND PIKULA, D.: Am. Rev. Tuberc., 1953, *67*, 808.

The methods used throughout the study have been described in detail elsewhere.[2] Mice infected intravenously with the Vallée strain of *M. tuberculosis* (bovine) were used

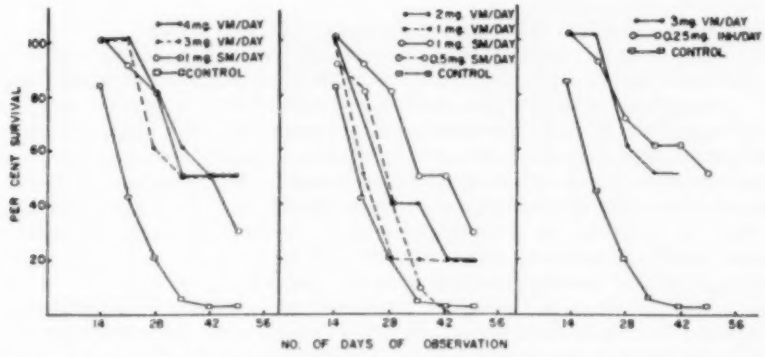

FIG. 2. Comparison of the antituberculous activities of viomycin, isoniazid, and streptomycin when administered twice weekly (Vallée strain: *M. tuberculosis*, bovine).

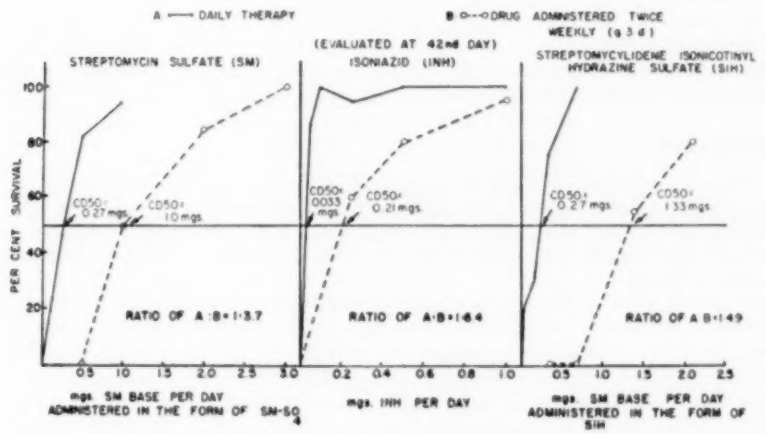
A DAILY THERAPY
B DRUG ADMINISTERED TWICE WEEKLY (q 3 d)
(EVALUATED AT 42nd DAY)
STREPTOMYCIN SULFATE (SM)
ISONIAZID (INH)
STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE SULFATE (SIH)
PER CENT SURVIVAL
RATIO OF A:B=1:3.7
RATIO OF A:B=1:64
RATIO OF A:B=1:49
mgs. SM BASE PER DAY ADMINISTERED IN THE FORM OF SM·SO4
mgs. INH PER DAY
mgs. SM BASE PER DAY ADMINISTERED IN THE FORM OF SIH

FIG. 3. Comparison of antituberculous activities of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine when administered daily and twice weekly (Vallée strain: *M. tuberculosis*, bovine).

in all instances. Treatment was started on the day of infection, the drug being administered subcutaneously in a single daily injection twice weekly, e.g., on Tuesdays and Fridays.

[2] HOBBY, G. L., LENERT, T. F., DONIKIAN, M., AND PIKULA, D.: Am. Rev. Tuberc., 1951, *63*, 17.

A minimum of 10 mice were used per dosage level[1]; in some instances 20 to 30 were used. The effect of treatment was evaluated on the basis of per cent of animals surviving after varying periods of time, and on the basis of average survival time, as compared with that of untreated control animals.

The results obtained in this study are illustrated in figures 1 and 2.

As indicated therein, streptomycin, viomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine all are capable of suppressing an acute tuberculous infection in mice when administered twice weekly in appropriate dosages. Under the conditions of these experiments, the degree of chemotherapeutic action observed with various dosages of these antituberculous agents indicated that isoniazid is three to six times more active than streptomycin, while streptomycin is approximately three times more active than viomycin. The action of streptomycylidene isonicotinyl hydrazine, on the other hand, is proportional to the amount of streptomycin and of isoniazid present in the amount of streptomycylidene isonicotinyl hydrazine used (figure 3).

Comparison of these data with those obtained in previous experiments in which treatment was carried out on five successive days weekly indicated that the individual daily dose comparable to the CD_{50} was 3.7- to 6.4-fold greater when the intermittent (twice weekly) regimen was used than when the daily regimen was used.

In summary: Streptomycin, viomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine were found to be effective when used intermittently in the control of experimental tuberculosis in mice. The individual daily dosage required, however, was significantly greater than that previously found effective when the drugs were administered on a daily basis.

Research Division
Chas. Pfizer & Co., Inc.
Brooklyn, New York.

Gladys L. Hobby
Tulita F. Lenert

April 20, 1953

[1] Dosages of streptomycin and viomycin are expressed in terms of the equivalent weight of streptomycin or viomycin base, respectively. Dosages of isoniazid and of streptomycylidene isonicotinyl hydrazine are expressed in terms of total weight. Streptomycylidene isonicotinyl hydrazine, in the form of the sulfate, is 70 per cent streptomycin base and 16 per cent isoniazid.

Correspondence

IODINE IN LEPROSY

To the Editor of The American Review of Tuberculosis:

In the December, 1952, issue of THE AMERICAN REVIEW OF TUBERCULOSIS, pp. 765–777, there is material which interests me particularly. For one thing, it is the first that I recall ever seeing in another periodical of the kind that I have repeatedly run in the *International Journal of Leprosy* as "symposia by correspondence," which our readers find of interest. Also interesting to me is the consensus that little is really known about the effects of iodine in tuberculosis.

I wonder if there would be any interest, in a collateral way, in the experience with iodine—specifically potassium iodide—in the treatment of leprosy. It was much used, first and last, over a period of many years.

To start with the book of Hansen and Looft (1894)—passing up Leloir, Babes, and others—which so far as treatment is concerned deals mostly with the experience of Danielssen many years before, it is said, "At the beginning of his studies Dr. Danielssen had great confidence in iodine, but he soon learned what a very dangerous remedy it was in this disease. Even small doses of iodine produce new eruptions of leprous tubers or patches, and Dr. Danielssen therefore ultimately used it as a test in cases of apparent cure. When a patient was considered cured, he gave him iodide of potassium, and if no new eruption developed, the cure was considered complete." They then go on to tell of brief experience with Unna's method of using the drug.

When Muir took charge of leprosy research in Calcutta he revived KI, the idea being —in a way of speaking—to stir up or mobilize the infection so that chaulmoogra could get at it. In that period some held as an objective to induce "lepra reactions"; and there was something in that, for mild degrees of reactions often seem beneficial, whether spontaneous or induced. The big trouble was that one could never tell what would come of the triggering off of a reaction. It might be mild, brief, and beneficial or severe, prolonged, and harmful. For example, during my long tour in 1931 I saw patients in South Africa, mentioned in one of my first studies of tuberculoid leprosy (Internat. J. Leprosy, 1934, *2*, 279), who had had only simple flat macules but after injudicious testing with KI had blossomed out spectacularly in long-persistent tuberculoid reaction. At Dichpalli, in India, Lowe showed me a number of cases of nerve abscesses which had, at least in part, resulted from reactions in tuberculoid cases induced by KI when Isabel Kerr, a devotée of Muir's, had been using it. He reported (Internat. J. Leprosy, 1934, *2*, 301) that about 2 per cent of some 5,000 cases which he had seen there had had such abscesses—an absolutely unparalleled incidence—and that about one-half of them had developed under KI treatment.

Many workers came to condemn its use, and the Leonard Wood Memorial Round-Table Conference wrote into its report (Philippine J. Sc., 1931, *44*, 449; reprinted in Internat. J. Leprosy, 1934, *2*, 329), "Some remedies may produce marked disturbances in the course of the disease, and their use may be dangerous in certain stages, while in others they may be beneficial. The use of iodides, especially potassium iodide, should be studied in this connection. Their employment, however, should be attended with extreme caution. . . ." Muir, who was still for KI at that time, was of course a member of that conference. Seven years later, however, the committee on therapy of the Cairo congress was more emphatic (J. Egyptian M. A. 1938, *21*, 149; Internat. J. Leprosy, 1938, *6*, 389): "With regard to

treatment with potassium iodide, the use of this drug is frequently followed by disastrous results. It is therefore to be discouraged for the purposes of diagnosis, treatment, or as a test of recovery unless in very skilled and experienced hands." Again Muir was a member of that committee, and I am confident that had he not been present the full stop would have been placed immediately after "test of recovery." That certainly was the view of many clinicians, and nowadays one rarely hears of the use of that drug. It is not mentioned in the report on therapy of the Havana congress (1948). Muir seems not to have abandoned the idea entirely, however, for in his *Manual of Leprosy* (1948) he says, in connection with the length of treatment with the sulfones: "Iodides have a specific effect in showing up concealed lepromatous foci. It is possible that their careful administration in cases that have become bacterioscopically negative may be of use in determining the length of further treatment required, and perhaps in speeding up the elimination of residual foci; but this matter still calls for very careful investigation." And, I would add, it still does!

If there is seen anything of interest in this peculiar effect of KI in leprosy, a "somewhat distant" relative of tuberculosis, and if present-day opinions of leading clinicians should be desired, one might write to Muir, Lowe, Davison, and Lauro de Souza Lima for statements. Their addresses are:

Dr. E. Muir, International Leprosy Association, 167 Victoria Street, London, S.W.1.
Dr. John Lowe, Nigeria Leprosy Service, Uzuakoli, Nigeria.
Dr. A. R. Davison, Westfort Institution, Pretoria, South Africa.
Dr. Lauro de Souza Lima, Sanatorio Padre Bento, Gopouva, São Paulo, Brazil.

Sincerely yours,
H. W. WADE

CULION SANITARIUM
PALAWAN, PHILIPPINES

March 24, 1953

TOXICITY OF ISONIAZID

To The Editor of the American Review of Tuberculosis:

This communication is concerned with a rather high incidence of peripheral neuritis in patients receiving higher doses of isoniazid than the usual 3 to 5 mg. per kg. per day.

In view of the demonstration that daily doses of 20 mg. of isoniazid per kg. are nontoxic for the rhesus monkey (Schmidt, L. H., Hoffmann, R., and Hughes, H. B.: Am. Rev. Tuberc., 1953, *67*, 798), we have explored the possibility of using such a dose in patients with tuberculosis. One hundred patients have been placed on doses of isoniazid varying from 6 to 24 mg. per kg. per day, along with the usual dose of streptomycin, 1 gm. either daily or twice weekly. Thirty-five of these had previously been on 3 to 5 mg. per kg. per day for periods ranging from five to fifty-one weeks. Of the 65 patients who had received no previous isoniazid, 11 (17 per cent) have developed peripheral neuritis. This occurred twenty-four to seventy-one days after the drug was begun. Considering only those patients who have received the high dose for at least three weeks, the incidence is 11 of 54, or 20 per cent. More instances of neuritis have appeared on the higher dose levels than on the lower so that, of 16 patients who received 16 to 24 mg. per kg. per day for at least three weeks, 6 (37 per cent) have developed neutritis.

Of the 35 patients who had been on the drug at the time the high dose was started, 22 (33 per cent) have developed neuritis. In these patients the neuritis generally appeared earlier, in some instances as quickly as six days after the dose was increased. It is inferred

that the previous medication exerted a partial toxic effect, allowing the toxic dose to make its clinical effect apparent sooner.

In 4 patients who received 20 mg. per kg. per day in whom blood levels of isoniazid were studied, the average was somewhat higher (10.6 γ per ml.) than in patients who received the same dose in which neuritis did not appear (7.1 γ per ml.).

The syndrome presented by these patients is fairly typical of polyneuritis in general. Initial complaints were numbness of the fingers, later of the hand, arm, or foot as well, occasionally with burning or tingling. Aching within the bones, and calf tenderness, appeared in some. The reflexes were not consistently changed but were markedly exaggerated or abolished in some. Vibratory sensation was not lost early in the course, in contrast to what is seen in other forms of neuritis, but disappeared in the more severe cases more consistently.

Thirteen of the 33 patients with neuritis have so far shown no improvement since the discontinuance of isoniazid for at least five weeks. Four have recovered completely, and varying degrees of improvement have appeared in the remaining 16. These results can not be correlated with the dose received or with the clinical condition of the patient. In one patient in whom clearing of the neuritis has occurred, isoniazid was re-started at 300 mg. per day, with no recurrence of neuritis. It is perhaps pertinent to note that the isoniazid used in these patients was furnished by two separate manufacturers.

It is believed that this rather important toxic effect of isoniazid should be brought to the attention of the readers of the REVIEW, since it appears to interfere with the use of this valuable drug when it is given in higher doses. A more detailed manuscript will be submitted later, when our current studies are more complete.

DUNHAM HOSPITAL AND THE CINCINNATI GENERAL HOSPITAL, CINCINNATI, OHIO

J. PARK BIEHL[1]
JOHN H. SKAVLEM

June 2, 1953

[1] Teaching Fellow American Trudeau Society.

Books

THE NATURE AND SIGNIFICANCE OF THE ANTIBODY RESPONSE. Edited by A. M. Pappenheimer, Jr. Pp. 227, Columbia University Press, New York, 1953, Price $5.00.

Between the making of new observations in the clinic and in the laboratory and the recording of such findings in standard texts, there exists often a significant temporal gap. Symposia such as this fifth one of a series organized by the Section on Microbiology of the New York Academy of Medicine make valuable additions to scientific literature in bringing together in single volumes the more recent results obtained by a number of investigators whose interests center on a given field.

During the past decade, there has been increasing interest in the role which immunologic processes may play in a variety of disease states. Essential to an understanding of such disorders is knowledge of the basic mechanisms concerned therein. In *The Nature and Significance of the Antibody Response*, seventeen authors have contributed fourteen essays on various aspects of this field. The papers are grouped to deal with three broad topics: the production of antibodies, the kinds of antibodies, and antigen-antibody reactions in the tissues. Without loss of unity, there is variation in the approach of the several authors to their subjects, some discussing the controversial aspects of a problem without partisanship, others favoring one of several points of view in still debated issues. The material is presented, however, in such a fashion as to be intelligible to those not intimately concerned with the technical aspects of immunology and immunochemistry but at the same time in such a way as to indicate trends in methodology as well as in thinking. Useful bibliographies are appended to each chapter. This work should be of service to all who are interested in immunology and its application to clinical medicine.

ROBERT AUSTRIAN
New York, New York

X-RAY DIAGNOSIS OF CHEST DISEASES. By Coleman B. Rabin. Pp. 142, The Williams and Wilkins Company, Baltimore, 1952, Price, $12.00.

This volume constitutes a verbatim reprint of material already published in Golden's Looseleaf *Diagnostic Roentgenology* and is therefore owned by or familiar to most radiologists. The author's contribution is unusually good in scope, detail, and organization. Rather than a brief survey of the subject, the book is a detailed, authoritative, occasionally repetitive discussion. Dr. Rabin has probably achieved more permanence for his book than often is accorded a publication in so changing a field as roentgenology. It is possible that the reprint will outlive its parent.

Criticism may be directed at the publisher. The paging system necessitated by the looseleaf nature of the original form of publication has been retained. This was presumably the result of an interest in economy and is to be condemned. Similarly, a reluctance to spend money has apparently led the publisher to print a list of errata, which is bound into the book facing the title page. Surely the slight expense necessary for the publisher (rather than the purchaser) to make seven minor changes could have been expected. The reproductions, when compared with those of the parent volume are noticeably lacking in quality, possibly as a result of their having become worn in the printing of the original volume. It is hoped that in future printings, the paging will be revised, the errors corrected, and certain of the illustrations replaced or re-engraved. These shortcomings

are attributable to publisher rather than author. The book is recommended to all physicians interested in diseases of the chest who do not already possess Golden's Looseleaf *Diagnostic Roentgenology*.

CHARLES T. DOTTER
Portland, Oregon

PRINCIPLES, PROBLEMS, AND PRACTICES OF ANESTHESIA FOR THORACIC SURGERY. By Henry K. Beecher. Pp. 65, Charles C Thomas, Springfield, Illinois, 1952, Price, $2.50.

Anesthesia in thoracic surgery is concisely and conservatively treated by Dr. Henry K. Beecher in his monograph, *Principles, Problems, and Practices of Anesthesia for Thoracic Surgery*. This monograph contains the basic fundamentals for the conduct and maintenance of anesthesia for operations in the thorax. Some of his views on particular aspects of this subject are still controversial, although his presentation concerning them is didactic.

The wealth of material presented by Dr. Beecher is drawn from thousands of operative cases. Therefore, he is very ably qualified to indicate the pitfalls into which the neophyte may fall. His outline of the conduct of specific types of surgery is excellent. He emphasizes three most important aspects to be carefully controlled—troublesome reflexes, obstructing secretions, and adequate ventilation. Attention to these fundamentals will provide adequate oxygenation, freedom from abnormal elevation of the carbon dioxide tension of the blood, and lessening of circulatory strain.

The present reviewer recommends this book to the embryo anesthesiologist and to all surgeons who enter the thoracic cavity.

The bibliography is small, but well chosen.

JOSEPH F. ARTUSIO
New York, N. Y.

THIS IS YOUR WORLD. By Harry A. Wilmer. Pp. 165, Charles C Thomas, Springfield, Illinois, 1952, Price, $5.50.

Any attempt to understand and ameliorate the difficulties in living of people who have tuberculosis is salutory and to be encouraged. Though Osler epitomized the attitude of a few physicians, saying that the doctor who would treat a person with tuberculosis well must know what is in his head as well as his chest, the number of worthwhile studies dealing with the peculiar and powerful emotional stresses of this disease has been, paradoxically, quite small.

Instead of positing a "tuberculosis personality," thereby alienating some workers and confusing many, Dr. Wilmer sets up for discussion situations of persistent tension staff-patient relationships; the vicissitudes of a rigid, dependent, authoritarian sanatorium environment; enforced idleness; misconceptions about tuberculosis. This approach, the harvest of the author's experience in group psychotherapy in a sanatorium setting, is implemented by recorded talks, the reading of a long narrative in dramatic form called *This Is Your World*, and a group of provocative dialogues—all of which are included in the book.

By no means a pat, didactic formulation, this book can most profitably be viewed as a theme from which the reader must develop his own variations. With this restriction it is a valuable aid to the internist or social worker, as well as to the psychiatrist, who is eager to use all weapons available to maintain and bolster psychological integrity and, finally, conquer tuberculosis. It is in a more general way a happy addition to the modest literature

concerned with the emotional problems of people who have diseases of a chronic or slowly healing nature.

S. L. WERKMAN
Charlottesville, Virginia

THE LITERATURE ON STREPTOMYCIN 1944–1952. By Selman A. Waksman. Pp. 553, Rutgers University Press, New Brunswick, New Jersey, 1952, Price, $5.00.

No laboratory or clinical investigator in the field of antituberculous therapy can fail to appreciate the great value of Dr. Waksman's bibliography of 5,550 papers dealing with all aspects of the study of the streptomycin drugs. The addition of 4,379 titles since the last edition of this book in 1948 attests the magnitude of the task so satisfactorily undertaken by Dr. Waksman. The expanded subject index contributes greatly to the usefulness of this volume.

No investigator studying tuberculosis nor any library dealing with this field can afford to be without this bibliography.

LAWRENCE B. HOBSON
New York, New York

BOOKS RECEIVED

TREATMENT OF RESPIRATORY EMERGENCIES INCLUDING BULBAR POLIOMYELITIS. By Thomas C. Galloway. Pp. 94, Charles C Thomas, Springfield, Illinois, 1953, Price, $3.00.

TEXTBOOK OF VIROLOGY. Second edition. By A. J. Rhodes and C. E. van Rooyen. Pp. 561, with illustrations, Williams & Wilkins Company, Baltimore, 1953, Price, $8.00.

PROCEEDINGS OF THE ANNUAL MEETING OF THE COUNCIL FOR HIGH BLOOD PRESSURE RESEARCH, 1952. AMERICAN HEART ASSOCIATION. Pp. 113, American Heart Association, New York, 1952, Price, $1.75.

MICROBIAL GROWTH AND ITS INHIBITION. Pp. 285, World Health Organization, Geneva, Switzerland, 1952, Price, $3.00.

WHAT EVERYONE SHOULD KNOW ABOUT TUBERCULOSIS. By M. Santosham. Pp. 239, Crystal Publishing House, Vepery, Madras, 1952.

CLASSIFICATION OF PULMONARY TUBERCULOSIS. By Milosh Sekulich. Pp. 322, with illustrations, William Heinemann, Medical Books, Limited, London, 1953.

ADJUSTMENT TO PHYSICAL HANDICAP AND ILLNESS: A SURVEY OF THE SOCIAL PSYCHOLOGY OF PHYSIQUE AND DISABILITY. By Roger G. Barker in collaboration with Beatrice A. Wright, Lee Meyerson, and Mollie R. Gonick. Pp. 440, Social Science Research Council, New York, 1953, Price, $2.00.

CONSIDERATIONS SUR LES FORMES LES PLUS COMMUNES DE LA TUBERCULOSE RECONTRÉES CHEZ L'INDIGENE AU CONGO BELGE. By G.-P. Lambrichts. Pp. 99, Leopoldville, Belgian Congo, 1952.

ANNUAL REPORT OF THE FEDERAL SECURITY AGENCY, 1952. PUBLIC HEALTH SERVICE. Pp. 89, Superintendent of Documents, U. S. Government Printing Office, Washington, 25, D. C., Price, 30 cents.

THE EPIDEMIOLOGY OF HEALTH. By Iago Galdston. Pp. 197, Health Education Council, 10 Downing Street, New York 14, N. Y., 1953, Price, $4.00.

REHABILITATION OF THE PHYSICALLY HANDICAPPED. Revised Edition. By Henry H. Kessler. Pp. 275, Columbia University Press, New York, 1953, Price, $4.00.

EPIDEMICS IN COLONIAL AMERICA. By John Duffy. Pp. 274, Louisiana State University Press, Baton Rouge, Louisiana, 1953, Price, $4.50.

PUBLIC HEALTH NURSING CARE OF THE SICK AT HOME. Pp. 57, Department of Public Health Nursing, National League for Nursing, 2 Park Avenue, New York, Price, $1.00.

BROMPTON HOSPITAL REPORTS. Volume XXI. Pp. 206, Gale & Polden Limited, Aldershot, England, 1952.

CHRONIC PULMONARY EMPHYSEMA. By Maurice S. Segal and M. J. Dulfano. Pp. 180, Grune & Stratton, New York, 1953, Price, $5.50.

DOCTORS BY THEMSELVES. An Anthology compiled by Edward W. Griffith. Pp. 614, The British Book Centre, Inc., New York, 1953, Price, $4.50.

BACTERIOSTATIC ACTIVITY OF 3500 ORGANIC COMPOUNDS FOR MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS. By Guy P. Youmans, Leonard Doub, and Anne S. Youmans. National Research Council, Washington, D. C., 1953, Price, $5.00.

The Toxicity of Isoniazid

A Report of the Committee on Therapy

The following report represents the opinion of the Committee on Therapy of the American Trudeau Society and is published at this time as a committee consensus with full realization that additional information gained with the passage of time may modify some parts statement.

The value of any drug in the treatment of any disease must be judged by the relationship between its therapeutic effectiveness and its tendency to cause toxic reactions. Cost and ease of administration are also important, but less so than the factors of effectiveness and toxicity. This report is an attempt to summarize the available information on the toxicity of isoniazid. The impression is that, while toxic reactions do occur, they are relatively minor in comparison with those due to streptomycin, dihydrostreptomycin, amithiozone, and viomycin.

Soon after the announcement of the value of isoniazid in the treatment of tuberculosis, the Committee on Therapy of the American Trudeau Society began to receive a few reports of serious and even fatal reactions to this drug. For this reason, it was decided to attempt to collect these reports, so that as rapidly as possible the medical profession might be given a comprehensive statement on the toxic potentialities of this drug. A letter was published in the American Review of Tuberculosis and several other medical journals, requesting that physicians submit to the Committee individual reports of untoward reactions that they had observed. In addition, the medical directors of several of the manufacturers of this drug were asked to notify the Committee of any toxic reactions which came to their attention. The Committee has been gratified by the cooperation it has obtained and would like to thank all the contributors (1) for their interest and help. In addition, several published reports (2) have been combined with the Committee's data. About 150 instances of toxic reactions have come to the attention of the Committee.

It is estimated that toxic reactions to isoniazid occur in about 5.0 per cent of the patients receiving the drug and that, in about 1.0 per cent of all patients, the reaction is serious enough to contraindicate further treatment with isoniazid. Fatal reactions clearly related to isoniazid have been extremely rare.

With few exceptions, the daily dose of isoniazid for adults was between 150 and 300 mg. and there was no evidence that the frequency of toxic reactions was affected by variations within this particular range. Limited experience with doses up to 600 mg. per day has not revealed any increase in toxicity. There was no suggestion that the reactions were related to the age, sex, or race of the patient. No evidence was obtained to confirm the previous suggestion that the concurrent administration of certain drugs (epinephrine, ephedrine, atropine, and demerol) might increase the toxicity of isoniazid.

Most of the reactions subsided when administration of the drug was stopped. In fact, some of the minor reactions gradually subsided even though administration of the drug was continued. Some of the patients whose treatment was interrupted were able to resume treatment later without difficulty.

Isoniazid is excreted through the kidneys, and cumulation of the drug in the body may occur when renal function is impaired. For this reason, the renal function should be

measured before the drug is given and, if the function is abnormal, the dose of the drug should be adjusted accordingly.

The nervous system was most frequently involved, the commonest reactions being twitching of muscles, restlessness of the extremities, and hyperreflexia. These reactions were not serious and frequently subsided even though treatment was continued. Other minor neurological reactions were nervousness, apprehension, insomnia, and headache. The more serious reactions affecting the nervous system were vertigo, syncope, convulsions, peripheral neuritis, major psychoses, and difficulty in micturition, requiring catheterization in a few instances. Convulsions were particularly prone to occur in patients with a history of a convulsive disorder, and such patients should be given isoniazid with great caution and with adequate anticonvulsant medication. The psychoses were more common in patients with an unstable personality and those with organic brain disease. They were very serious in some instances, requiring transfer to a mental disease hospital.

Allergic reactions were next most common, drug fever being the most frequent reaction, sometimes preceded by a chill. Dermatitis, usually a fine, red pruritic rash, was also reported. Dyspnea, sometimes serious and even fatal, was apparently produced by bronchospasm. These reactions usually subsided when administration of the drug was stopped and recurred when treatment was resumed. There was some suggestion that desensitization by administration of small doses might be effective, but further study is needed.

Except for constipation, gastrointestinal reactions were not common. Those reported were nausea, vomiting, abdominal cramps, and diarrhea. Results of liver function tests were fairly frequently abnormal, but became normal when the drug was discontinued. A few cases of clinical jaundice were reported. The kidneys were affected in a few cases, producing severe albuminuria, cylindruria, and edema.

The hematopoietic system was also affected in a few instances, the commonest reaction being purpura. One patient had thrombocytopenia, but the exact mechanism was not determined in the others. Abnormal bleeding has been manifested by hemoptysis, hematuria, and rectal bleeding. Several cases of leukopenia with agranulocytosis have been observed.

Painful swollen joints developed in a few patients. In one child, who also had purpura, a bloody joint effusion developed.

It is impossible to be certain that the above reactions were all due to isoniazid, but the evidence available suggested a causal relationship. For this reason, it would seem wise for any clinician prescribing this drug to observe his patients carefully for such reactions. In many instances, it may be possible either to continue treatment in spite of the reaction or to resume treatment after a brief interruption. When the more serious reactions occur, it would seem wise to abandon treatment with isoniazid and, if possible, administer another of the antituberculous drugs. Neither is the above list thought to be complete. Undoubtedly, as more and more patients are treated with this drug, other less common reactions will be noted and will have to be added to the results of this survey.

Iproniazid

Toxic reactions to iproniazid were similar in character but occurred much more frequently than they did following administration of isoniazid, and fatalities have been reported somewhat more often. In addition, patients treated with iproniazid for as long as six months frequently experienced so-called withdrawal symptoms, consisting of vivid terrifying dreams, head consciousness, nervousness, restlessness, depression, anorexia, nausea, and vomiting, when the drug was discontinued. Such symptoms may persist for

two to four weeks and may be quite disturbing to the patient unless forewarned. Because of this greater toxicity, iproniazid is still available for experimental use only.

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NOTES

POSTGRADUATE COURSE IN ASUNCION-PARAGUAY

A postgraduate course in tuberculosis for physicians was held at the Bella Vista Sanatorium, Asuncion-Paraguay, June 5 to 20, 1953. The course was under the direction of Dr. Juan Max Boettner, director of the sanatorium, and was sponsored by the Public Health Department, the Inter-American Cooperative Public Health Service, and the World Health Organization. The course covered both the epidemiological and clinical aspects of tuberculosis.

JAMES ALEXANDER MILLER FELLOWSHIP

The New York Tuberculosis and Health Association announces that the James Alexander Miller Fellowship for Research in Tuberculosis will be available from July 1, 1954 to June 30, 1955.

The Miller Fellowship is designed to support a qualified medical investigator who will devote full time to a research project with a definite bearing on tuberculosis. Assurance must be provided that the applicant will be acceptable in the laboratory or clinic of his choice; and that he will be provided with the facilities necessary for the pursuit of the work. The annual stipend will be $5,000.

Applications should be submitted in duplicate by October 1, 1953. Application forms will be supplied on request. Address The New York Tuberculosis and Health Association, 386 Fourth Avenue, New York 16, N. Y. Awards will be announced December 1, 1953.

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY—1954

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of abstracts of scientific papers for presentation at its next annual meeting in Atlantic City, New Jersey, May 17 to 21, 1954.

Full information can be obtained by writing to the Chairman of the Medical Sessions Committee, American Trudeau Society, 1790 Broadway, New York 19, N. Y.

THE AMERICAN REVIEW OF TUBERCULOSIS

ABSTRACTS

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CLINICAL STUDIES, TUBERCULOSIS

Treatment of Bronchopleural Fistula.—Postoperative bronchopleural fistula continues to be frequent after resection for tuberculosis. There was a mortality rate of 30 per cent among 30 patients who developed such a fistula between 1945 and 1950. Death was usually due to pulmonary or extrapulmonary spread of the disease. The mortality rate decreased to 16.6 per cent among 15 patients who were treated by a modified technique. When a fistula was diagnosed, it was considered to be an emergency. The objectives were threefold: (*1*) drainage of the pleural space, (*2*) eradication of the space by re-expanding the remaining lung or by thoracoplasty, and (*3*) closure of the fistula itself. In most patients, drainage was by means of open thoracotomy, which immediately lessened the chance of spread. As soon as the patient's condition permitted, thoracoplasty was attempted. Frequent blood transfusions were necessary during this period. An average of five or six operative procedures, including partial scapulectomies and Schede thoracoplasties, were necessary before the pleural space was obliterated. When the space had been reduced to a narrow sinus, the tracts were delineated with radiopaque solutions and it was decided whether to attempt plastic closure with a muscular graft, to attack the bronchial defect directly, or to be satisfied with a living patient who had a small draining sinus and infrequently positive sputum. Results with plastic closure and muscle grafts were not encouraging. In a few patients there was apparent early success, but most subsequently failed. During the past year the initial attack was more conservative. As soon as a fistula was diagnosed, a large catheter attached to a pump was inserted through an intercostal space. This took care of the immediate emergency and gave time to determine whether the air was leaking from the bronchial closure or from a raw surface left as a result of segmental or lobar separation. Limited thoracoplasty was then done on 5 patients. The fistula closed, the sputum remained negative, and the wound healed in each patient. Early open thoracotomy and excision of a remaining lobe or segment of a lobe were done in 3 patients with complete success. Early open thoracotomy and suture of the fistula were done in 2 patients, but both fistulas reopened and both patients died. Emphasis should be placed on efforts to avoid bronchopleural fistula by means of careful case selection and preoperative preparation. Chemotherapeutic agents should be administered continuously for a long enough period before operation to stabilize the lesion. —*The complications and the results of treatment of bronchopleural fistula following resection for tuberculosis, J. D. Murphy, B. B. Becker, & H. V. Swindell, J. Thoracic Surg., December, 1952, 24: 578.*—(*R. E. MacQuigg*)

Band-like Infiltrates.—In the course of chronic tuberculosis there is frequently a radiographic picture which mimics interlobar effusion. The so-called juxta-fissural band infiltrate casts a dense shadow at the base of the upper or middle lobe and is sharply delimited below by the fissure. Pneumothorax or pleuroscopy demonstrates the lesions to be within the lung. They always occur in association with one or more cavities superior to them, either in close proximity, separated by an area of healthy lung, or occasionally high in the contralateral lung. A careful search, including tomography, is often neces-

sary to demonstrate the cavity which may be small and inconspicuous. The infiltrates appear to be dependent upon the cavities above them and represent either tuberculous bronchial dissemination or atelectasis secondary to bronchial obstruction from an embolus originating in the cavity. They should not be considered as representing extensive caseation destined to progress to total necrosis; they usually clear quite readily with antibiotic treatment. They tend to recur, however, if the cavity remains open. Thus, treatment should be primarily directed against the responsible cavity.—*Infiltrats en bande juxta-scissuraux au cours de la phtisie tertiare et leur signification pathologique, J. Brun & L. F. Perrin, Poumon, December, 1952, 8: 809.*—(*F. S. Lansdown*)

Plastic Sponge Prosthesis After Resection.—In 50 cases of lobectomy, pneumonectomy, and segmental resection, polyvinyl sponge (Ivalon) was used as an intra- and extrapleural plombage. Even if it is advisable to insert the sponge at a second operation, it is simpler and less shocking than a thoracoplasty. Among 200 resections for tuberculosis during the preceding year, 48 per cent were of the segmental type. Before that year approximately 95 per cent of more than 400 resections were lobectomies or pneumonectomies. No foreign substance placed in the body is ideal and each has disadvantages. If these are minimal, as demonstrated in this small group of cases, the procedure can be recommended.—*Plastic sponge prosthesis following resection in pulmonary tuberculosis, J. W. Gale, A. R. Curreri, W. P. Young, & H. A. Dickie, J. Thoracic Surg., December, 1952, 24: 587.*—(*R. E. MacQuigg*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Sarcoid and Sarcoid-like Granulomas.—Pathologically, sarcoidosis is characterized by epithelioid cell tubercles with little or no necrosis and by the frequent presence of refractile or apparently calcified bodies in the giant cells of the tubercles. The characteristic lesions may be replaced by fibrosis, hyalinization, or both. An attempt was made to clarify the relationship among the sarcoid and sarcoid-like granulomas from 27 necropsied cases examined at the Armed Forces Institute of Pathology. The cases were selected from the following groups: (*1*) with undiagnosed noncaseous granulomas, 13 cases; (*2*) with granulomas of sarcoidosis, 10 cases; (*3*) the same as (*2*) but with pathologic evidence of secondary tuberculosis, 4 cases. The ages of the patients ranged from 18 to 68 years. Seven of the 27 patients were Negroes. The granulomas were found in a variety of organs and tissues. Although there was considerable overlapping, the cases were now divided into four groups as follows: *Group I* (2 cases) showing asteroid bodies and large plate crystal slits within giant cells; *Group II* (12 cases) with only asteroid bodies within giant cells; *Group III* (7 cases) with asteroid and Schaumann bodies within giant cells; *Group IV* (6 cases) with Schaumann bodies and nonlipid crystalline material within giant cells. The first three groups were considered as related wholly or in part to local or systemic disturbances in lipid metabolism. The cases in *Groups III* and *IV* were morphologically most compatible with the definition of sarcoidosis advanced by the National Research Council. In both of these latter groups the underlying disease was sometimes complicated by tuberculosis, much as silicosis is frequently complicated by tuberculosis. It is apparent that the lesions of sarcoid and sarcoidosis are difficult to define because they form a spectrum of granulomas.—*Sarcoid and sarcoid-like granulomas: A study of twenty-seven post-mortem examinations, R. L. Engle, Jr., Am. J. Path., January–February, 1953, 29: 53.*—(*J. S. Woolley*)

Mediastinal Sarcoidosis.—A young man complained only of weight loss and fatigue. He had no palpable adenopathy or splenomegaly but roentgenography, including tomography, revealed enormous masses on either side of the trachea and mediastinal border, one of them nearly the size of an orange.

Repeated cultures and guinea pig inoculations of his sputum and gastric contents failed to demonstrate tubercle bacilli. Intradermal tuberculin was negative to 1:10 dilution. Bronchoscopy showed extrinsic compression of the trachea. Pulmonary arteriography showed the opaque substance to stop abruptly at the right brachiocephalic trunk after its confluence with the subclavian vein. A tentative diagnosis of Boeck's sarcoid was made and a trial of chemotherapy was instituted. Streptomycin, 1 gm., and *p*-aminosalicylic acid, 12 gm., were given daily for sixty days. By the end of this time the huge mediastinal masses had disappeared from sight on both standard films and tomography. Bronchoscopy revealed no abnormality, and the intradermal skin test had become frankly positive to tuberculin. The patient had gained 11 kg. and was entirely asymptomatic. Arteriography now showed normal vascularization. The author points out that the finding on venography confirms Bariéty's observation that arterial obstructions are more apt to be due to malignant tumors and venous obstructions to benign ones.—*Maladie de Besnier-Boeck-Schaumann à type ganglio-médiastinal pur avec compression réversible du système cave supérieur, etudiée angiographiquement: Action remarkable de l'association streptomycine-P.A.S., C. Scarinci, Poumon, December, 1952, 8: 825.*—(*F. S. Lansdown*)

Failure in Chronic Lung Disease.—The effects of infection and bronchial obstruction in producing acute anoxia were studied in several cases of chronic pulmonary disease, representative of the pulmonary insufficiency due to emphysema seen in everyday practice. On clinical examination, this group had inadequate bellows motion of the chest and bronchial obstruction secondary to chronic bronchial infection. These factors as well as poor distribution of gases resulted in alveolar-respiratory insufficiency. The resultant chronically impaired alveolar ventilation was characterized by arterial anoxia, hypercapnia, and sometimes a low arterial pH. Such patients repeatedly have acute bronchitis and bronchopneumonia, which usually respond to antibiotic therapy. Occasionally, bronchial obstruction due to the infection is so severe that it diminishes the effective alveolar ventilation enough to cause acute anoxia and carbon dioxide retention. This, in turn, may precipitate cardiac failure. The final step occurs when such a patient breathes a high oxygen concentration. Relief of anoxia, the stimulus to ventilation operating through the carotid receptor mechanism, causes further retention and respiratory acidosis. Sound therapy must provide an adequate airway and improve the effective alvelolar ventilation. Besides antibiotics, bronchodilators such as epinephrine aerosol are helpful. Oxygen therapy is necessary and should be instituted immediately, but increasing mental stupor or confusion, a decrease in ventilation, a significant increase in the arterial pCO_2, or a decrease in the pH indicate a need for prompt artificial respiration. The latter increases effective alveolar ventilation, promotes bronchial drainage, and maintains a more adequate airway. The Drinker-Collins respirator or the pneumonic balance resuscitator serve the same purposes in cooperative or comatose patients. If means for artificial respiration are not available, oxygen should be used intermittently as a compromise. Cardiac failure may respond to this regimen even without the use of digitalis. This drug, however, should be employed when indicated.—*Precipitation by pulmonary infection of acute anoxia, cardiac failure and respiratory acidosis in chronic pulmonary disease, D. J. Stone, A. Schwartz, W. Newman, J. A. Feltman, & F. J. Lovelock, Am. J. Med., January, 1953, 14: 14.*—(*T. H. Noehren*)

Cause of Acute Pericarditis.—The records of 96 cases of acute pericarditis diagnosed in a general hospital during a sixteen-year period were reviewed with special regard to the causative agents involved. Rheumatic fever accounted for 40.6 per cent; purulent pericarditis, 19.8 per cent; tuberculous pericarditis, 7.3 per cent; benign nonspecific pericarditis, 10.4 per cent uremia, 11.5 per cent;

neoplasm, 3.1 per cent; and the so-called collagen diseases, 2.1 per cent of the 96 cases. There remained 5.2 per cent of the total which could not be attributed to any cause. Patients in the rheumatic and pyogenic groups complained most frequently of fever, chest pain, and dyspnea. In the tuberculous group, cough, weakness, and epigastric pains were common, whereas chest pain was rare. Pleural effusion accompanied tuberculous pericarditis more commonly than the other forms.—*The cause of acute pericarditis, R. L. Reeves, Am. J. M. Sc., January, 1953, 225: 34.—(W. J. Steininger)*

Bone Coccidioidomycosis in Children.—In 26 cases of coccidioidomycosis of bone in children, the lesions were osteolytic, similar to those in adults, and either single or multiple. Some patients had an accompanying pulmonary lesion or cutaneous abscess. As a rule, the process was chronic with a relatively good prognosis for eventual recovery. Parenchymal infiltration of the lung frequently preceded the skeletal lesion and, by the time bone was involved, only residual pulmonary adenopathy remained in some cases. In endemic areas the lesions are not uncommon, and elsewhere they are frequent enough to warrant consideration of coccidioidomycosis when there are osteolytic lesions in children. A history of travel or previous residence in an endemic area may suggest this diagnosis.—*Coccidioidomycosis of bone in children, J. Dykes, J. K. Segesman, & J. W. Birsner, Am. J. Dis. Child., January, 1952, 85: 34.—(M. J. Small)*

Löffler's Syndrome Following Penicillin.—A case of Löffler's pneumonia developed subsequent to a course of penicillin therapy. That penicillin may have been the etiologic agent is suggested by the clinical course and the results of the skin testing (Author's summary).—*Loeffler's syndrome following penicillin therapy, S. Reichlin, M. H. Loveless, & E. G. Kane, Ann. Int. Med., January, 1953, 38: 113.—(T. H. Noehren)*

Neonatal Hyaline Disease of Lungs.—Radiographic studies in hyaline disease of the lung in the newborn infant heretofore have not been reported previously. In one case the clinical picture was that of asphyxia and the gross morphology of the lung suggested atelectasis. The radiograph, however, showed the lung fields unusually well aerated. This aeration was shown microscopically to be due to air in the terminal bronchioles and alveolar ducts, and not in the alveoli, which were collapsed. Moreover, a hyaline membrane over the terminal bronchioles and alveolar ducts probably prevented adequate interchange of gases. Demonstration of good aeration of the lungs on the radiograph, paradoxically coupled with labored breathing and other clinical manifestations of asphyxia, does not occur in asphyxia neonatorum due to other causes. It may constitute a method of diagnosing hyaline disease of the lungs but more cases must be observed before a conclusive statement can be made.—*The radiographic appearance of hyaline disease of the lungs in the newborn, I. Meschan, H. N. Marvin, V. H. Gordon, & G. Regnier, Radiology, March, 1953, 60: 383.—(W. J. Steininger)*

Causes of Fetal and Neonatal Death.—An adequate anatomic cause of death was demonstrated in 107 of 137 fetuses and infants who died during the neonatal period. Intrauterine anoxia, as evidenced by aspiration of excessive amounts of the contents of the amniotic sac, was the single leading cause of death. Intrapulmonary hyaline membranes were the leading cause of death in liveborn infants. The pathogenesis of these intrapulmonary hyaline membranes is not entirely clear; there is evidence that vascular damage and increased capillary fragility play a role. Less frequent causes of death were intraventricular hemorrhage, bronchopneumonia, congenital syphilis, intracranial trauma, kernicterus, intrauterine anoxia, and inflammatory lesions. Histologic examination of multiple sections of the lungs is an essential part of any post-

mortem examination of a fetus or newborn infant. In this series, this type of examination alone would have indicated the cause of death in 35 per cent of the fetuses and infants. Further studies are necessary for a better understanding of the pathogenesis and significance of many of the commonly observed pulmonary lesions.—*Causes of fetal and neonatal death with special reference to pulmonary and inflammatory lesions, J. B. Arey & J. Dent, J. Pediat., February, 1953, 42: 206.*—(*M. J. Small*)

Atelectasis.—It would seem logical that chronic atelectasis should be attacked more vigorously by conservative measures before it reaches the stage which requires radical surgical measures. The use of trypsin appears to offer this possibility since it has a mucolytic action. In 2 cases inhaled trypsin relieved chronic atelectasis due to bronchial and bronchiolar obstruction in patients with bulbar poliomyelitis. Further experience should be accumulated in carefully selected patients.—*Atelectasis: Physiopathology and treatment, M. E. Peck & S. Levin, J. Thoracic Surg., December, 1952, 24: 619.*—(*R. E. MacQuigg*)

Chronic Pulmonary Artery Thrombosis.—The clinical syndromes of (*1*) acute obstruction of the pulmonary arteries by emboli or thrombosis, and (*2*) chronic obstruction related to certain degenerative and inflammatory diseases of the heart, lungs, or pulmonary vessels are well known. There is a third type of pulmonary artery obstruction characterized by slowly developing thrombosis of the pulmonary arterial tree with progressive right ventricular failure. The pathogenesis in a proved case may be difficult or impossible to establish. One study concluded that most thrombi in the pulmonary arteries are embolic, originating in the systemic veins or the right side of the heart. Various pulmonary, cardiac, and pulmonary vascular diseases, however, may cause local thrombus formation in the pulmonary artery and its branches. These are: (*1*) primary lung diseases, such as infection or tumor, which directly involve the pulmonary arterial tree and lead to pulmonary thrombosis with propogation of the clot; and (*2*) diffuse vascular diseases of the lungs which usually are secondary to degenerative or chronic inflammatory disease or are secondary to mitral stenosis but occasionally result from primary vascular inflammation. Here the following events occur: (*a*) pulmonary hypertension, (*b*) hypertrophy of the right ventricle, (*c*) right heart failure, and (*d*) thrombosis of the pulmonary artery, which aggravates the existing disease and results in sudden death. The symptoms of this sequence are often those of ordinary congestive failure. Associated pulmonary disease may be apparent. The diagnosis, therefore, is seldom made before death, the formation of thrombi usually being unsuspected. The duration of symptoms from the onset of thrombosis or embolism may be as short as two weeks or as long as four years. The occurrence of thrombi is commonest in the older age groups, but instances have been reported in children and, in the presence of antecedent emboli, chronic massive thrombosis may occur at any age. Cyanosis may or may not be present, although it is sometimes severe. Pulmonary infarction or embolism is not a necessary part of the clinical or pathologic picture, although it may occur if fragments of the thrombi become detached and migrate peripherally into the smaller branches of the pulmonary arteries; embolism of the lung may characterize the clinical onset. The roentgenographic changes result from ischemia of the lung peripheral to the intravascular occlusion and are manifested by diminished vessel markings in those parts of the lung fields supplied by the affected arterial branches. These peripheral vessels are contracted and relatively devoid of blood distal to the point of obstruction. They are small and few, producing the net roentgenographic effect of segmental blackening of the lung fields. When embolism or thrombosis involves a major branch, the affected vessels

will often be enlarged at the point of obstruction and dilated proximally to it, providing a clue to the pathologic process. At the same time, the roentgenographic appearance of pulmonary infarction may occur. In patients who survive long enough, there may be progressive enlargement of the hilar arteries and simultaneously progressive blackening of the lung fields due to ischemia. In addition, the hilar arteries may become more sharply outlined because they no longer possess intrinsic pulsations, at times resembling large lymph nodes.—*Chronic massive thrombosis of pulmonary arteries, D. R. Keating, J. N. Burkey, H. K. Hellerstein, & H. Feil, Am. J. Roentgenol., February, 1953, 69: 208.*—(*T. H. Noehren*)

Enzymatic Aerosol for Obstructive Atelectasis.—A 4-year-old child suffering from amyotonia congenita had an intercurrent bilateral pneumonia and atelectasis of the left lower lobe. Prolonged treatment with penicillin and terramycin was ineffective. Due to the systemic disease the patient was unable to cough forcibly and was too weak to breathe deeply or to inflate balloons. Streptokinase-streptodornase aerosol therapy was unsuccessful. Crystalline trypsin, 250 mg. dissolved in 12 cc. of Sorensen's phosphate buffer solution, was administered by nebulizing 3 cc. every thirty minutes for four doses. Following this treatment, expansion of the collapsed lung occurred within a few hours. Intrabronchial instillation of the appropriate proteolytic enzyme in conjunction with suction might result in even more complete removal of blocking exudate; in this case bronchoscopic aspiration was considered dangerous due to the hazard of general anesthesia in amyotonia congenita. The use of trypsin or streptokinase and streptodornase by aerosol route merits further trial.—*Treatment of obstructive atelectasis by aerosol administration of proteolytic enzymes, J. F. Craven, J. Pediat., February, 1953, 42: 228.*—(*M. J. Small*)

Pulmonary Retention of Droplets.—The retention of triphenyl phosphate droplets in the human respiratory tract was measured when the flow rate of the air sample was 60 liters per minute; the droplet size and the average time spent in the lungs was varied. The percentages retained were higher at this flow rate than at lower rates, other factors being equal. The curves of percentage retention as a function of the particle size were similar at the two flow rates. There was minimal retention of particles approximately 0.4 γ in diameter. The percentage retention increased from 20 to 60 per cent as the mean time in the lungs increased from two to twelve seconds.—*Retention of air-borne particulates in the human lung: III, H. D. Landahl et al., A. M. A. Arch. Indust. Hyg. & Occup. Med., December, 1952, 6: 508.*—(*T. H. Noehren*)

Abnormal Respiratory Responses to "Curare" Drugs.—In a series of 2,800 patients who received various "curare" drugs, 13 persons demonstrated respiratory inadequacy at the end of operation. Apnea, lasting 40 to 140 minutes occurred in 6; prolonged respiratory depression for 70 to 130 minutes was noted in 7. There was no apparent relationship between these unexpected respiratory effects and the age or sex of the patient, or the anesthetic agents administered. Apnea occurred significantly more often in the patients whose preoperative physical status was less adequate.—*Abnormal respiratory responses to various "curare" drugs during surgical anesthesia: Incidence, etiology, and treatment, R. D. Dripps, Ann. Surg., February, 1953, 137: 145.*—(*M. J. Small*)

Cortisone for Berylliosis.—A patient with beryllium granulomatosis developed severe respiratory symptoms eight years following exposure. Treatment with cortisone resulted in dramatic symptomatic improvement although the findings in chest roentgenograms were not materially affected. The response was maintained for eighteen months on a daily dose of 25 mg. of cortisone by mouth without significant untoward reactions. The literature contains 3 reports on a total of 7 cases of beryllium granulomatosis which had

responded to corticotropin but none in which cortisone was effective.—*Beryllium granulomatosis: Report of a case showing response to cortisone, J. J. Robbins & W. F. Lyons, Ann. Int. Med., January, 1953, 38: 120.*—(*T. H. Noehren*)

Circulatory Arrest.—In thoracotomy or operations in the upper abdomen, the heart should be massaged within three minutes of the time the diagnosis of cardiac arrest is made, allowing a maximum of one minute to establish the diagnosis. A defibrillator must be at hand for electric shock. Epinephrine, 1:1,000, and a syringe with a long, wide-bore needle should always be ready. As soon as the diagnosis is established, the needle is inserted into the heart. The wide bore allows rapid aspiration of blood to demonstrate that the needle is in one of the cardiac chambers. One milliliter is injected. If this is done quickly enough and the heart is still beating slowly, an immediate response is noted and the needle can be removed. If a few seconds after the first injection no response is noted, 5 ml. are injected. An immediate response usually occurs. If it does not, 10 ml. are given. During these injections, only one minute is lost. In the meantime artificial positive pressure ventilation is instituted through an intratracheal tube. With this scheme, regular rhythm was reestablished in 4 successive cases; in 2 the recovery was complete, but in 2 others the injection was too late and cerebral damage had already occurred. The value of intracardiac epinephrine has been underestimated because the beating heart of experimental animals reacts badly to epinephrine. An accidental intravenous injection of 0.5 ml. of 1:1,000 epinephrine killed a healthy 20-year-old woman. Most surgeons use only 1 ml., which is too little and often is without effect. When 10 ml. of 1:1,000 epinephrine was accidentally injected in a patient's heart at standstill, it immediately responded with complete recovery and no untoward effects. The heart in standstill is anoxic, the reaction is delayed, and a large initial dose is needed. There is no untoward effect once the heart beats, as the action of epinephrine is too brief. A combination of epinephrine and 5 ml. of 1 per cent procaine may be valuable. Speed is all-important and epinephrine alone is preferred if some seconds are gained by omitting the procaine. In many bilateral tuberculous cases the pathologic changes of lungs and pleura make it impossible to open the thorax and perform cardiac massage within three minutes. Due to the diminished respiratory reserve, many tuberculous patients do not tolerate an unexpected thoracotomy. In 2 such cases, large doses of epinephrine given intracardially saved the patients. More patients are saved by immediate cardiac injection of large doses of epinephrine than are lost by cardiac massage one minute too late or by ventricular fibrillation.—*Circulatory arrest, V. O. Bjork, J. Thoracic Surg., December, 1952, 24: 611.*—(*R. E. MacQuigg*)

Rupture of Heart During Cardiac Massage.—Aortic embolectomy was accomplished in a 69-year-old woman with hypertensive, arteriosclerotic heart disease. Cardiac arrest occurred during closure of the abdominal wall. Attempted cardiac resuscitation was complicated by rupture of the right ventricle. (Authors' summary).—*Rupture of the heart during cardiac massage, E. S. Hurwitt & B. Seidenberg, Ann. Surg., January, 1953, 137: 115.*—(*M. J. Small*)

Diagnosis of Pulmonary Metastases.—Because there is more widespread use of chest roentgenograms and increasingly radical surgery, more cases of pulmonary metastases are being diagnosed. A roentgenographic diagnosis of pulmonary metastases may be difficult or impossible in certain cases. Serial films are helpful; biopsy of available tissue may be necessary. The appearance and configuration of the pulmonary lesions offer little aid in determining the site of the primary disease. When pulmonary metastases are suspected but the original lesion remains silent or obscure, the organs most likely to be involved should be investigated first. In a

series of 105 cases of pulmonary metastases, the site of the primary tumor was as follows: genitourinary tract, 42, with cancer of the breast accounting for 21 of this group; gastrointestinal tract, 11, with carcinoma of the colon in 7 cases; lymphoblastoma, 9; skin and melanoma, 9; respiratory tract, 5; soft tissues, 8; miscellaneous, 9; primary unknown, 12. The origin of pulmonary metastases will remain unsolved in a certain percentage of cases even after a thorough clinical investigation and exhaustive necropsy. This is especially true in those cases with widespread metastatic deposits or when the cell type is highly undifferentiated. Follow-up studies of cancer cases should always include films of the lung, especially in the presence of respiratory symptoms. Although pulmonary metastases may be found in any age group, the incidence rises sharply after 40 years and is highest in persons more than 60 years of age.—*Diagnosis of pulmonary metastases, P. E. Russo & C. J. Cavanaugh, Radiology, February, 1953, 60: 198.*—(*W. J. Steininger*)

Bronchogenic Carcinoma.—A series of 151 bronchogenic carcinomas were studied from the standpoint of their early symptomatology and the techniques employed in their early diagnosis. The tumors may be divided into two groups: (*1*) those originating in the larger tracheobronchial system, namely, the trachea, the main bronchi, the lobular bronchi, and the orifices of the segmental bronchi, that is, the bronchogenic cancer visible bronchoscopically; (*2*) the carcinomas originating in the smaller bronchial ramifications, which may be termed parenchymatous cancer. These two types of cancer, in their early stages, behave like distinct entities with different symptoms and each requires its own diagnostic technique. The early symptoms of the first or bronchial type, which comprises 45 per cent of the cases, are those of bronchial irritation with cough and mucoid expectoration but without radiographic lesions. Subsequently, there are canalicular obstruction, radiographic shadows of segmental distribution, and pulmonary infection. Bronchoscopy is the preferred diagnostic method for this bronchial lesion, since it can be seen and biopsied in all cases. A negative bronchoscopy in a patient with a segmental pulmonary process excludes bronchial neoplasm. The second or parenchymatous type, which includes 55 per cent of the cases, does not give obstructive symptoms. It grows asymptomatically in the pulmonary parenchyma until it excavates, invades such neighboring organs as the pleura, large bronchi, or mediastinum, or produces either distant metastases or systemic symptoms. It is visible radiographically while still small but it is then asymptomatic and is detected early only by routine roentgenographic examinations. In this group of cancers the only reliable diagnostic measure is exploratory thoracotomy. Other techniques such as bronchoscopy, bronchography, sputum examination, and puncture biopsy give uncertain or indirect results in almost all cases.—*Diagnóstico del carcinoma broncogenico incipiente: Analisis de 151 observaciones, J. Carlos Dighiero, Hoja tisiol., December, 1952, 12: 369.*—(*F. Perez Pina*)

Alveolar Cell Carcinoma.—In each of 5 cases of so-called primary alveolar cell tumor of the lung and a probable sixth case, the tumor was malignant, producing regional or distant metastasis and involving both lungs. The tumor was multiple nodular in type in 3 cases and combined nodular and diffuse in the other 3, but the histologic structures of the tumors were identical. In structure and behavior the tumors resembled carcinoma, and they are believed to have arisen from terminal bronchiolar epithelium. Such tumors have been called cancerous pulmonary adenomatosis. The unique histologic structure of tall cuboidal cells lining the pulmonary alveoli, usually as a single layer, with a gently serrated border, and occasionally with papillary projections into the alveolar spaces, the characteristic gross differences from other pulmonary neoplasms, and the constant roentgenologic picture serve to place this tumor in a separate category.—*Primary alveolar cell carcinoma of the lung, J. H. Fisher & W. J. Holley,*

A.M.A. Arch. Path., February, 1953, 55: 162.—(E. Bogen)

Identification of Pulmonary Cancer Cells in Smears.—The cytologic techniques of examining smears of sputum and bronchial washings now allow approximately accurate estimates of the type of malignant pulmonic tumors from which cells have exfoliated. This affords a basis for prognosis as well as diagnosis. A series of smears from 341 cases of pulmonary cancer was chosen to include only cases in which the cytologic diagnosis was conclusive or fairly conclusive of malignant neoplasm and in which sections of surgical or post-mortem material were available. The type of tumor diagnosed from the smears was checked against the results of the histologic examination. The accuracy of the diagnosis as to the presence of pulmonary carcinoma was found to be approximately 90 per cent. The percentages of the various types accurately diagnosed by cytology were: epidermoid carcinoma, 89.9; pleomorphic carcinoma, 69.2; adenocarcinoma, 81.4 for all types, 78.4 for primary growths; anaplastic carcinoma, 83.5; and all other types, 0. Smears of sputum were found to be more reliable for this purpose than those from bronchial aspirates.—*The identification of types of pulmonary cancer in cytologic smears, N. C. Foot, Am. J. Path., November–December, 1952, 28: 963.—(J. S. Woolley)*

Mediastinal Lymphosarcoma.—A primary lymphosarcoma of the mediastinum in a 7-month-old infant rapidly disappeared following 1,360 r of roentgen therapy. The child has been asymptomatic and apparently cured for the past twelve years. The diagnosis of lymphosarcoma is not a hopeless one. Apparent cures do occur following prompt and adequate therapy (Authors' summary).—*Mediastinal lymphosarcoma in an infant: Twelve-year survival following radiation therapy, M. H. Stich, J. Rubinstein, A. B. Friedman, & M. Morrison, J. Pediat., February, 1953, 42: 235.—(M. J. Small)*

Hemangioma of the Mediastinum.—Neoplasms of vascular origin in the mediastinum are rare. To date, 18 have been described, of which 7 were hemangiomas. To this latter group is added the case of an 18-year-old white woman who complained of nonradiating epigastric pain associated with anorexia, nausea, and occasional vomiting but who had no symptoms related to the vascular or respiratory systems. Her physical examination was negative. Chest roentgenograms revealed an anterior mediastinal mass extending into the right hilar area. Kymography showed that the tumor did not pulsate. Laminagraphy revealed a homogenous, discrete, round, soft tissue mass and a separate smaller mass below the body of the tumor. Angiocardiography demonstrated a pressure defect on the superior vena cava and associated displacement of this vessel posteriorly. Thoracotomy revealed a grayish, somewhat cystic mass measuring 7 by 8 by 8 cm. in the anterior mediastinum on the right side. This had a tendency to bleed easily. Below and continuous with the body of the tumor there was a nodular extension which was the smaller tumor seen on the laminagrams. In one area, the mass appeared to invade the right side of the heart. There were extensions into the left hilum as well as the subdiaphragmatic areas. It was impossible to remove the lesion. Biopsy specimens consisted of a hemangioma with benign characteristics histopathologically but grossly and microscopically invasive. Radiation therapy had no effect on the size of the mass. Seven months after operation the patient was asymptomatic.—*Hemangioma of the mediastinum, J. M. Keegan., Am. J. Roentgenol., January, 1953, 69: 66.—(T. H. Noehren)*

Lobectomy for Bronchiectasis.—At Fitzsimons Army Hospital 237 consecutive patients with bronchiectasis have been treated by single lobectomy during the period January 1, 1946 through May 31, 1952. The diagnosis was based principally upon the history and bronchographic findings. All were confirmed by gross and microscopic study of

the removed lobe. Approximately 20 per cent had bronchographic evidence of residual bronchiectasis in a few or several remaining bronchopulmonary segments after lobectomy. Blood loss during and for the first twenty-four hours after operation, as determined by a study of blood volume using Evans blue dye, averaged 1,760 cc. Patients were, with rare exceptions, returned to military duty and are effectively carrying out all types of assignments. Of the 237 patients, only one died of massive pulmonary embolism on the fifteenth day, a mortality rate of 0.42 per cent. The major complications of bronchopleural fistula and empyema occurred in 3 and 6 per cent, respectively, of the patients. The decreasing operative mortality for bronchiectasis places it in a category lower than most intra-abdominal surgical operations, and hesitancy in recommending it, when indicated, is not justified.—*Lobectomy for bronchiectasis, J. H. Forsee & P. E. J. Klinger, Ann. Surg., January, 1953, 137: 74.*—(*M. J. Small*)

Esophageal Obstruction Due to Mediastinal Paraffinoma: Surgical Reconstruction.—Six years after paraffin oil was introduced into the extrapleural space of a patient, she developed dysphagia due to a paraffinoma of the mediastinum. This paraffinoma invaded the esophagus to produce a stricture which proved refractory to prolonged attempts at dilatation. A pedicle graft of colon was brought up into the left chest, the proximal end was passed up into the neck through the thoracic inlet and anastomosed to the cervical esophagus, the distal end was anastomosed to the esophagus above the diaphragm. Five months after the operation the patient was perfectly well and had gained 20 pounds in weight. There was an occasional audible gurgle on swallowing, but no other difficulty. On one occasion she had a brief attack of abdominal pain and tried to vomit, which proved to be impossible.—*Esophageal obstruction due to paraffinoma of mediastinum: Reconstruction by intrathoracic colon graft, F. G. Kergin, Ann. Surg., January, 1953, 137: 91.*—(*M. J. Small*)

Traumatic Rupture of Thoracic Aorta.—Traumatic rupture of the thoracic aorta is rare, the diagnosis being established at autopsy in the majority of cases. Death, which is due to exsanguination, usually occurs rapidly following injury. The patient reported here survived ten days, eventually dying with a massive left hemothorax. The initial finding was that of a large left hemothorax with the mediastinum shifted to the right. Following aspiration of the left hemothorax, there was widening of the superior mediastinum in the region of the aortic knob. In retrospect, this was a mediastinal hematoma which, had its significance been recognized, might have led to surgical repair of the aortic tear. The trauma leading to thoracic rupture generally occurs from rapid deceleration associated with a sudden rise in intra-aortic pressure. In the case reported here the patient was a passenger in an automobile which smashed into another car. Autopsy revealed a complete transection of the aorta just distal to the left subclavian artery. The patient probably survived for ten days because of the false aneurysm which formed at the rupture site.—*Traumatic rupture of the thoracic aorta: A case report, W. H. Kastl, Ann. Surg., January, 1953, 137: 111.*—(*M. J. Small*)

Duplication of Alimentary Tract.—At least 28 cases of abdominal and thoracic duplication of the alimentary tract have been reported since 1946. Half of these were wholly or partially in the thorax. The cystic type is much more common in the chest but the tubulocystic type occurs there. Cysts may be of any size; the tubulocystic variety may extend from high in the neck to the diaphragm. They are invariably mediastinal, usually posterior to the midcoronal plane of the thorax. They are fixed by the prevertebral and mediastinal tissues, derive their blood supply from adjacent vessels, and have no true serosa of their own. Most of them lie posteriorly on the right, though a few have

occurred in the anterior half of the mediastinum and a few on the left. Anomalies of the cervical and upper dorsal spine, particularly spina bifida and hemivertebra, are frequently present also. The clinical signs are those of compression, intracystic pressure, peptic ulceration with perforation into bronchi and vessels, chest pain, cough, dyspnea, and hemoptysis. There may be symptoms of acute or chronic lung infection, such as pneumonia, bronchiectasis, and pleural effusion, or bronchial obstruction and pressure erosion with cough and sputum. Roentgenoscopy, 30-degree oblique films, and heavily penetrated anteroposterior Bucky films should help to outline the lesion. Laminagraphy may also aid. The appropiate decubitus film demonstrates a gas-fluid level or may be of particular value owing to the relative mobility of the heart as compared with the duplication. With the patient in the lateral recumbent position the former may drop away, exposing the relatively fixed duplication. Bronchography probably has limited value and may serve to differentiate between cystic pulmonary lesions and cystic duplications. Films of the spine and thoracic skeleton to demonstrate skeletal anomalies may also demonstrate erosion of ribs or vertebrae. Esophograms frequently demonstrate compression or displacement of that organ and may show a communication or double lumen. If a communicating duplication is suspected, a method should be devised to deliver contrast substance under slight pressure, in a steady flow, into the lumen of the segment of the suspected communication. This can be accomplished best by intubation of the esophagus, colon, or small intestine. In one case the extent of the lesion was demonstrated when the patient swallowed a large amount of barium which passed rapidly into and through the small intestine. The resultant rise in pressure within the gut lumen at the point of communication was enough to force a small amount of barium into the communicating tract, confirming the diagnosis of communicating alimentary duplication whose thoracic component penetrated the right leaf of the diaphragm.—*Transdiaphragmatic duplication of the alimentary tract, J. J. Snodgrass, Am. J. Roentgenol., January, 1953, 69: 42.*—(*T. H. Noehren*)

Anastomosis of Bronchus to Trachea.—The right bronchus was completely ruptured by external trauma to the patient's chest in an automobile accident. In addition to other injuries the patient sustained multiple right rib fractures and right pneumohemothorax. Bronchoscopy performed four weeks after the injury showed complete occlusion of the right bronchial orifice at the level of the carina and it was believed that complete bronchial rupture had occurred with sealing of the main stem orifice. Forty-six days after injury the right pleural space was freed of massive old blood clots as well as heavy fibrinous and fibrous bands, a loculated empyema cavity was evacuated, and the lung decorticated. The lung was found to be collapsed to a small oval approximately 6 cm. in diameter and held against the pericardium by a dense fibrous membrane. The occluded bronchus was aspirated and anastomosed to the trachea. Postoperatively, the lung expanded well, bronchoscopy showed good healing at the site of anastomosis, bronchospirometry detected only moderately reduced function of the right lung, and bronchograms revealed an essentially normal bronchial pattern. The most useful procedure in the diagnosis of bronchial rupture is bronchoscopic examination, and the treatment of choice is surgical repair of the injury.—*Anastomosis of right bronchus to trachea forty-six days following complete bronchial rupture from external injury, W. Weisel & R. J. Jake, Ann. Surg., February, 1953, 137: 220.*—(*M. J. Small*)

Congenital Diaphragmatic Hernia in Newborn.—A 22-hour-old infant was successfully operated upon for diaphragmatic hernia. Most infants with diaphragmatic hernia die in the first few days of cardiac or respiratory difficulty and should be operated upon immediately. A newborn infant with a sunken abdomen should be suspected of having a diaphragmatic hernia. The suspicion is con-

firmed by roentgenograms. The use of barium is rarely necessary and may make the surgery more difficult. Cyanosis should not be a deterrent to operation. The operative approach should be through the abdomen, and phrenic crush is not considered necessary.—*Congenital diaphragmatic hernia in the newborn: Review of the literature and report of a case, S. H. Tolins, Ann. Surg., February, 1953, 137: 267.*—(*M. J. Small*)

Traumatic Right Diaphragmatic Hernia.—Traumatic right diaphragmatic hernias are extremely rare, only 10 cases having been previously reported in the American literature. In the present case, the liver, ascending and transverse colon, and the stomach entered the right chest cavity through a traumatic diaphragmatic hernia. The transthoracic approach is preferable in repairing such hernia.—*Traumatic right diaphragmatic hernia with evisceration of stomach, transverse colon, and liver into the right thorax, J. W. Neal, Ann. Surg., February, 1953, 137: 281.*—(*M. J. Small*)

Space-Occupying and Ventilatory Air.—Sudden occlusion of a segmental bronchus does not ordinarily produce atelectasis; the air content of the occluded segment is maintained by a drift of air through the alveolar spaces from the adjacent nonobstructed segments. The occluded segment, however, slowly fills with mucoid secretions and the condition is recognized clinically and radiographically as segmental collapse. Thus, the air passages within a bronchopulmonary segment are terminal structures in so far as mucous secretion is concerned, but are not terminal with respect to air. Among patients diagnosed clinically as having bronchiectasis, bronchographic studies sometimes disclose quite a different pattern from the usual ending in saccular or cystic spaces; instead the dilated bronchi terminate abruptly at some distance from the visceral pleura and a zone of aerated lung surrounds the cylindrically dilated bronchi. The dilated bronchi extend into a zone of air-containing lung but in no way communicate with it. They have in fact lost their function as ventilating tubes. In diseased segments, the bronchi of which showed such changes, it was observed at the operating table that these segments could not be deflated even by gentle external pressure. Indeed, they did not deflate when excised and allowed to stand with widely open bronchial apertures. The air was trapped in the alveoli. It is concluded from these studies and those of other workers that there is an obliteration of the finer air passages distal to the secretory zone and the lining membrane and that the alveoli in this area are not atelectatic but emphysematous with the air replenished by collateral air drift from adjacent normal segments. Diseased lobes or segments of this kind receive an abnormally large flow of arterial blood from the systemic circuit through bronchial and mediastinal arteries. The greatly increased bronchial artery circulation may, by the resulting higher pressure gradient, block in whole or in part the shunt of venous blood that otherwise would occur. The architectural design of the lung is such that its spatial integrity tends to be preserved even after a morbid insult has destroyed the function of segmental structural units. This mechanism involves what is referred to as the space-occupying function of air within an organism. In an interpretation of the morbid anatomy and function of the lung, the space-occupying volume of air is an important consideration apart from the obvious exchange of air that is ventilation.—*The architectural basis of pulmonary ventilation, E. D. Churchill, Ann. Surg., January, 1953, 137: 1.*—(*M. J. Small*)

Function Studies in Bronchial Asthma.—Pulmonary function studies were performed in 42 patients with chronic bronchial asthma at a time when they were symptomatic. The results may be summarized as follows: (*1*) The vital capacity ranged between 51 and 158 per cent of the predicted normal value. The inspiratory capacity/vital capacity ratio

averaged 68 per cent. (*2*) The maximum breathing capacity ranged between 11 and 130 per cent of predicted normal value. (*3*) The resting ventilation was generally increased and ranged between 3.11 and 7.52 liters per minute per square meter of body surface. (*4*) The index of intrapulmonary mixing ranged between 1.0 and 12.12 per cent nitrogen. (*5*) The total lung capacity averaged 125.8 per cent of predicted normal value. The residual volume/total lung capacity ratio ranged between 35 and 67 per cent and averaged 47.0 per cent. (*6*) The maximum expiratory velocity averaged 3.9 liters per second (Author's summary).—*Pulmonary function studies in bronchial asthma: I. In the control state, J. A. Herschfus, E. Bresnick, & M. S. Segal, Am. J. Med., January, 1953, 14: 23.*—(*T. H. Noehren*)

Function Studies in Treated Bronchial Asthma.—Pulmonary function studies were performed in 42 patients with chronic bronchial asthma in the asymptomatic state after treatment which consisted of aminophyllin, 0.5 gm. intravenously, or neosuprel bronchodilator, 6 inhalations by aerosol sprays. Pulmonary function studies performed after treatment gave the following results: (*1*) The average improvement in the vital capacity was 10.6 per cent after aminophyllin and 16.9 per cent after bronchodilator aerosols. (*2*) The average improvement in the maximum breathing capacity was 30.9 per cent after aminophyllin and 50.0 per cent after bronchodilator aerosols. (*3*) The average improvement in the index of intrapulmonary mixing was 32.7 per cent after aminophyllin and 28.8 per cent after bronchodilator aerosols. (*4*) The average residual volume decreased slightly. The average total lung capacity did not change appreciably after treatment. (*5*) The average improvement in the residual volume to total lung capacity ratio was 7.4 per cent after aminophyllin and 8.1 per cent after bronchodilator aerosols (Author's summary).—*Pulmonary function studies in bronchial asthma: II. After treatment, J. A. Herschfus, E. Bresnick, & M. S. Segal., Am. J. Med., January, 1953, 14: 34.*—(*T. H. Noehren*)

Water-Soluble Contrast Medium.—Bronchograms obtained with viscous umbradil, a water-soluble contrast medium, are usually of good quality and are often superior to those obtained with an oil medium. The water-soluble medium has the advantage that it tends to adhere to the bronchial mucosa better than oil. It also mixes readily with the bronchial secretion, whereas oil frequently separates into droplets. The greatest advantage is the rapid disappearance of the medium from the lung, permitting early surgery without the risk of postoperative complications, particularly atelectasis. The present water-soluble medium, however, is not yet perfect. It has a more immediate local irritating effect than oil media and necessitates a more extensive and tedious anesthesia, making its use more time-consuming. Sufficient anesthesia may be obtained, however, without overdosage of the anesthetic.—*Bronchography with water-soluble contrast medium, E. Poppe & H. Ødegaard, Radiology, December, 1952, 59: 831.*—(*W. J. Steininger*)

Bronchography in Lobar Collapse.—Although lobar and segmental pulmonary collapse or other disease may usually be localized by ordinary roentgenologic means, there are many occasions when bronchography may give confirmatory and additional information. In other instances, chronic collapse may not be suspected until after bronchographic study. In the presence of collapse of a lobe, the remainder of the lung on the affected side will ordinarily undergo compensatory expansion in order to fill in the pleural dead space. The bronchi in the overexpanded lung undergo directional change, especially a "fanning around" movement, as can be demonstrated bronchographically. The most striking and confusing bronchial rearrangement occurs in the left lower lobe following collapse or lobectomy of the left upper lobe. The bronchial rearrangements which result from collapse of the other lobes are not as

pronounced or confusing as in the left upper lobe. With the increasing frequency of lobar and segmental resection of lung, roentgenologists are more and more apt to be confronted with distorted bronchial patterns in instances in which postoperative bronchograms have been made. The patterns following lobectomy closely resemble those resulting from collapse of the corresponding lobe (Author's summary). *Bronchography in chronic lobar collapse, R.C. Boyer, Am. J. Roentgenol., January, 1953, 69: 28.—(T. H. Noehren)*

ANTIMICROBIAL THERAPY

Intrathecal Isoniazid for Tuberculous Meningitis.—Tuberculous meningitis was proved in a 20-year-old woman who was comatose and had cranial nerve signs. She was treated with intrathecal doses of 200 mg. of isoniazid daily for eleven days. She also received oral isoniazid, 300 mg. daily; streptomycin intramuscularly, 2 gm. daily initially; and oral *p*-aminosalicylic acid, 9 gm. daily. Except for the intrathecal medication, the treatment was continued for four months. There was complete clinical recovery at this time and the abnormal cerebrospinal fluid findings had returned to normal. She left the hospital against advice after four months. Although apparently cured, the follow-up period is too short to allow any definite conclusion as to the permanence of this recovery.—*La isoniacida intrarraquídea a altas dosis en el tratamiento de la meningitis tuberculosa: A propósito del primer caso tratado; nota previa, Hoja tisiol., December, 1952, 12: 396.—(F. Perez Pina)*

Action of Streptomycin in Tuberculous Meningitis.—A new theory on the mode of action of streptomycin in tuberculous meningitis explains the rise of cerebrospinal fluid sugar after treatment with streptomycin. The hypothesis is formulated to correlate known clinical observations and laboratory data: Streptomycin in tuberculous meningitis causes inhibition of carbohydrate metabolism of the injured cells. The increase in spinal fluid sugar noted after the initial treatment with streptomycin is secondary to decreased utilization of glucose by the living cell and unimpaired flow of spinal fluid (Authors' summary).—*Mode of action of streptomycin in relation to the changes in spinal fluid sugar in tuberculous meningitis: III. A theory of the mode of action of streptomycin in vivo with a commentary on the current literature, R. H. Friedman, D. L. Thurston, & A. F. Hartmann, J. Pediat., February, 1953, 42: 171.—(M. J. Small)*

Cerebrospinal Fluid Sugar in Treated Tuberculous Meningitis.—Streptomycin intrathecally causes a rise in the cerebrospinal fluid sugar in the initial treatment of tuberculous meningitis. This rise is not always correlated with clinical improvement in the disease process. The increase in cerebrospinal fluid sugar is true glucose and is not due to streptomycin's action as a nonglucose-reducing substance. After treatment with streptomycin and further progression of the disease process, variable results in spinal fluid sugar are obtained after intrathecal streptomycin (Authors' summary).—*Mode of action of streptomycin in relation to the changes in spinal fluid sugar in tuberculous meningitis: II. Observations on the effect of intrathecal streptomycin treatment on the cerebrospinal fluid sugar in tuberculous meningitis, R. H. Friedman, D. L. Thurston, A. F. Hartmann, H. Wolf, D. Minikis, & R. B. Gerstung, J. Pediat., February, 1953, 42: 161.—(M. J. Small)*

Effect of Streptomycin on Cerebrospinal Fluid Sugar.—Since 1948 there have been reports that the rise in cerebrospinal fluid sugar after intrathecal therapy with streptomycin in tuberculous meningitis did not indicate increasing glucose concentrations but was due to the chemical action of streptomycin as a reducing agent. The present experiments showed that streptomycin's reducing action does not invalidate "true glucose" determinations *in vitro* by the Shaffer-Hartmann method when the concentrations of the antibiotic are within the range attained

by the intramuscular and intrathecal administration of streptomycin. On the other hand, the Hagedorn-Jensen method does not appear to be reliable for determining true sugar in the presence of streptomycin.—*Mode of action of streptomycin in relation to the changes in spinal fluid sugar in tuberculous meningitis: I. The role of streptomycin as a reducing agent and the specificity of the Shaffer-Hartmann method for determining "true glucose" when this antibiotic is used (in vitro experiments)*, *R. H. Friedman, A. F. Hartmann, H. Wolf, M. Morton, & R. B. Gerstung, J. Pediat., February, 1953, 42: 157.*—(*M. J. Small*)

Healed Tuberculous Meningitis.—A case of tuberculous meningitis was clinically healed after treatment with streptomycin administered in divided doses, 3 gm. intramuscularly and 0.2 gm. intrathecally daily for eighteen days, followed by smaller doses until a total of 244.5 mg. was given. Almost four years later the patient died of adrenal insufficiency, and the pathologic examination confirmed the cure of the tuberculous meningitis. The leptomeninges were not thickened except for a small area in the interpeduncular region. Numerous cross sections of the brain failed to reveal abnormalities and microscopic sections confirmed the gross findings. The adrenal glands were almost entirely replaced by a caseous process in which there was no evidence of activity. The kidneys each contained a multiloculated cavity lined by yellowish-gray necrotic material without evident active tuberculosis.—*Morphology of healed tuberculous meningitis following streptomycin therapy, S. M. Jacobson & R. C. Greene, Am. J. Med., January, 1953, 14: 152.*—(*T. H. Nochren*)

Altering Virulence of Tubercle Bacilli.—It has been postulated by Dubos that a surface constituent of virulent tubercle bacilli is concerned in their pathogenicity. This "cord factor" was extractable only from cord-forming strains of tubercle bacilli and caused the characteristic parallel arrangement of the bacilli. Removal of the "cord factor" usually reduced or destroyed virulence. To test the validity of the above hypothesis, tubercle bacilli were cultured in the presence of Tween 80 and amithiozone. The former, a detergent, in proper dosage seemed to release the "cord factor" into the surrounding medium; the latter, not a detergent, appeared to inhibit the formation of the "cord factor." *M. tuberculosis* H37Rv was grown in the presence of concentrations of Tween 80, ranging from 0.05 to 2.1 per cent. Equal numbers of viable bacteria from these cultures were injected into mice, the average survival time serving as a criterion for the virulence of the bacilli. The bacterial virulence was reduced slightly in bacterial suspensions from cultures with 0.05 to 1.0 per cent of Tween 80 and considerably from cultures with 2.1 per cent Tween. These relatively avirulent bacteria gave rise to the same number of colonies, *in vitro*, as bacteria grown in ordinary media; their oxygen uptake was even greater, indicating a high degree of viability. The "cord factor" could be recovered from the culture filtrates containing high concentrations of Tween. The bacilli from such cultures decolorized methylene blue, while virulent organisms from the same strain did not. Reduction of methylene blue is typical of nonvirulent tubercle bacilli. Essentially the same changes occurred when virulent tubercle bacilli were grown in the presence of 0.5 γ of amithiozone per ml. This low concentration of the drug did not prevent the growth or reduce the number of viable bacilli in the culture; it reduced their virulence considerably and rendered them capable of reducing methylene blue. No "cord factor" could be recovered from the amithiozone cultures, suggesting that this drug interferes with the formation of the "cord factor" normally associated with virulence.—*Studies on the virulence of tubercle bacilli: Variations in virulence effected by Tween 80 and thiosemicarbazones, H. Bloch & H. Noll, J. Exper. Med., January 1, 1953, 97: 1.*—(*J. S. Woolley*)

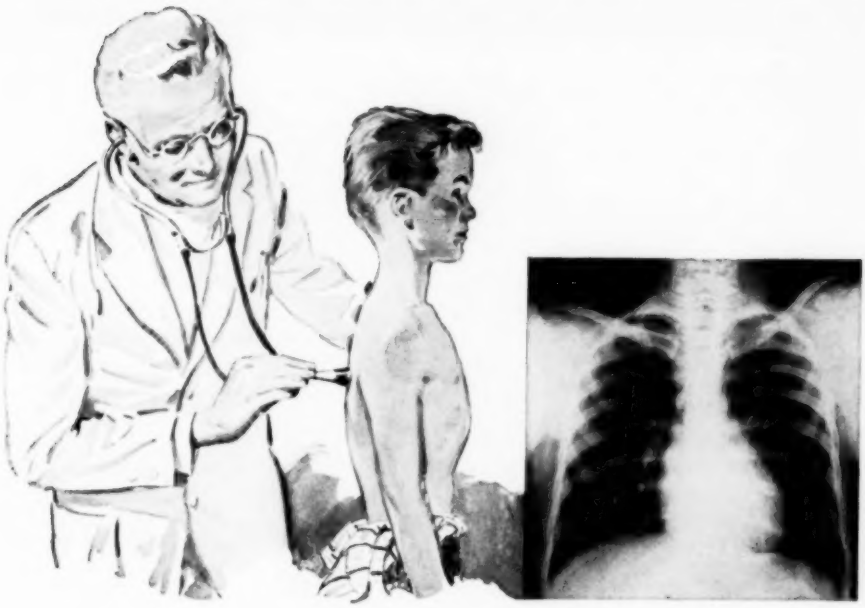
Resistance to Isoniazid.—The minimal bacteriostatic concentration of isoniazid varies

with different strains of tubercle bacilli. It was 0.0001 γ per ml. for the virulent strain H37Rv, 0.01 to 0.001 γ per ml. for the virulent strain H512, 25 γ per ml. for the nonpathogenic SAPC strain, and 1 to 10 γ per ml. for the 607 strain of paratuberculosis. Thus, pathogenic strains are more sensitive to isoniazid than are those of low or no virulence. The maximal concentrations of isoniazid compatible with growth of H512 were determined in 13 transfers during a period of seventy-eight days. Five phases are observed: *phase 1* (28 days, 4 transfers), 0.001 γ per ml.; *phase 2* (23 days, 5 transfers), 0.005 to 0.02 γ per ml.; *phase 3* (9 days, 2 transfers), 0.5 to 5 γ per ml.; *phase 4* (10 days, 1 transfer), 125 γ per ml.; *phase 5* (8 days, 1 transfer), 500 γ per ml. For the avirulent strain SAPC, resistance increases considerably after the fourth transfer (one month); maximal concentrations permitting growth increase rapidly from 75 γ to 200 and 500 γ per ml. The minimal bacteriostatic dose of isoniazid for *M. tuberculosis* H37Rv resistant to streptomycin is 0.0001 γ per ml. as in nonresistant strains. *M. tuberculosis* H512, which has been made resistant to 125 γ of isoniazid per ml., acquires resistance to streptomycin corresponding to a minimal bacteriostatic dose of 10 γ of streptomycin per ml., as compared with 0.1 γ of streptomycin per ml. for isoniazid-resistant strains.—*La résistance acquise du Mycobacterium tuberculosis a l'égard de l'isonicotinhydrazide (INH), C. Levaditi & J. Henry-Ereno, Ann. Inst. Pasteur, December, 1952, 83: 800.*—(*V. Leites*)

Antituberculous Agents in Vivo.—Guinea pigs were infected intradermally with 0.5 mg. of virulent tubercle bacilli of the Dupray S strain. The 30 animals were divided into 5 groups receiving respectively: (*1*) streptomycin, 42 mg. per kg.; (*2*) streptomycin, 30 mg. per kg.; (*3*) *p*-aminosalicylic acid (PAS), 300 mg. per kg. orally and 400 mg. per kg. parenterally; (*4*) isoniazid, 1.7 mg. per kg.; and (*5*) no treatment as controls. Treatment was instituted immediately after infection and pursued for twenty-one days. The results were evaluated by the extent of macroscopic involvement of the skin, lymph nodes, and spleen. Isoniazid had an extremely high bacteriostatic capacity, and complete protection against experimental tuberculosis was achieved with the small dose used. Streptomycin in the higher dosage achieved complete protection; there was partial protection with the dose of 30 mg. per kg., allowing small lesions of skin and lymph nodes. PAS gave only partial protection.—*Protection par la streptomycine, l'INH, et le PAS du cobaye tuberculisé par voie intradermique, R. Galland & J. Maillet, Ann. Inst. Pasteur, December, 1952, 83: 803.*—(*V. Leites*)

LABORATORY STUDIES

Pharyngo-Laryngeal Swabs.—A group of 100 patients was studied to compare the percentage of positive cultures for tubercle bacilli obtained by directly swabbing the pharynx and larynx and the percentage of positive results from conventional direct smears and cultures of the sputum and gastric washings. The swabs of the pharynx and the larynx were treated with sulfuric acid and with sodium hydroxide before culturing on Löwenstein-Jensen and Petragnani-Sáenz media. Of the 100 cases, 46 were negative by both pharyngo-laryngeal and conventional techniques; 42 were positive by both techniques; 10 were positive by the pharyngo-laryngeal technique but negative by the control techniques; and 2 cases were negative by the pharyngo-laryngeal technique but positive by the control techniques. The pharyngo-laryngeal technique is rapid, causes little discomfort to the patient, and is somewhat more reliable than the conventional techniques. It deserves more widespread application.—*El cultivo del hisopado faringo-laringeo en la investigación del bacilo de Koch, Abelardo Sáenz (h), Hoja tisiol., December, 1952, 12: 400.*—(*F. Perez Pina*)



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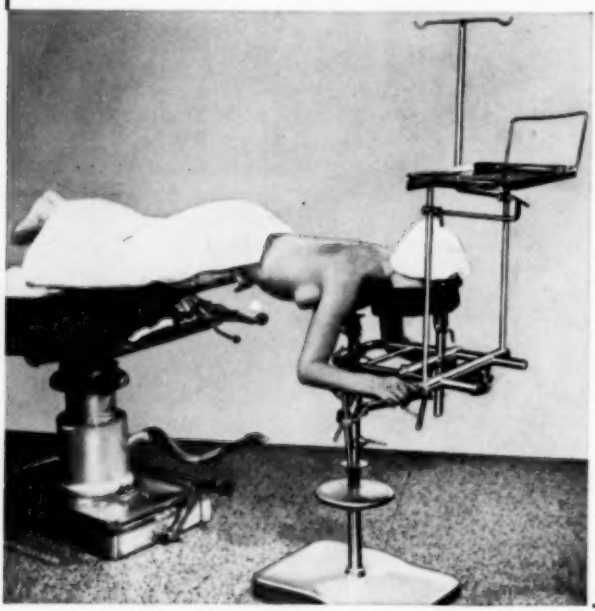

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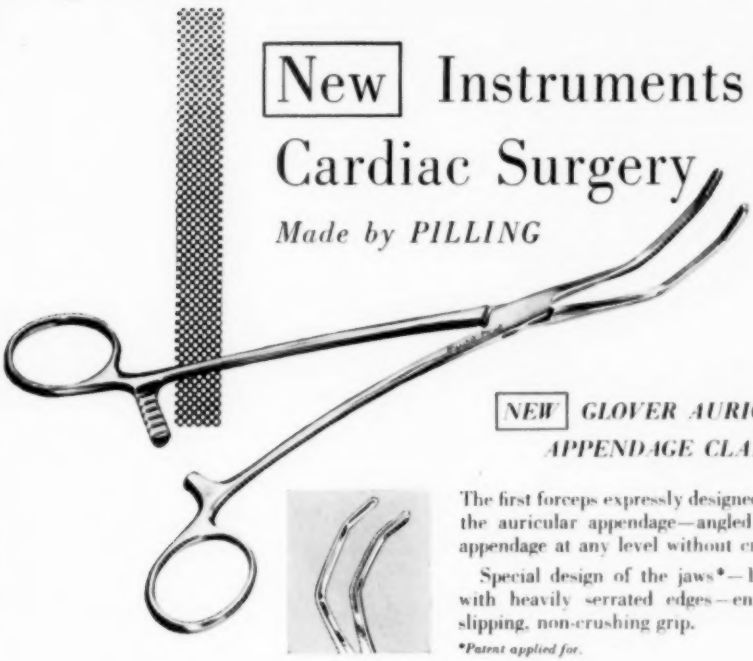

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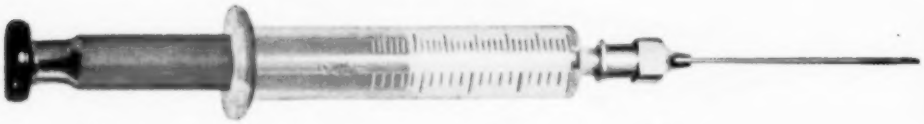

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